Doctoral Thesis for the degree of Doctor of Philosophy, Faculty of Food Science and Nutrition

Lifelong nutrition and bone health among the elderly

Validity of a food frequency questionnaire on intake at different periods of life, and the association between lifelong consumption of milk and cod liver oil and hip bone mineral density in old age.

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Age is an issue of mind over matter. If you don't mind, it doesn't matter.
Mark Twain

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Abstract

Background: Few studies exist on the validity of food frequency questionnaires (FFQs) administered to elderly people. An FFQ on dietary intake during three different periods of life, adolescence (14-19y), midlife (40-50y) and current old age, was developed for the Age/Gene Environment Susceptibility (AGES) Reykjavik Study. Assessing the validity of the AGES-FFQ is important for its use in studies on diet-related disease risk and health outcomes, as incorrect information may lead to false associations between dietary factors and diseases.

Milk is an important source of calcium and cod liver oil is a traditional source of vitamin D in Iceland. Consumption of both milk and cod liver oil is recommended for people of all ages in Iceland, not the least for the sake of bone health. However, whether and to what extent, lifelong consumption is associated with bone mineral density (BMD) in old age, the time of greatest risk of osteoporotic fractures, is unclear.

The aim of the present thesis was to assess the validity of questions of the AGES-FFQ on midlife and current consumption. Furthermore, to assess the association between lifelong consumption of milk and cod liver oil and hip BMD in old age among participants of the AGES-Reykjavik Study. Also, the association between current intake of cod liver oil and serum 25(OH)D was assessed.

Methods: Data was gathered from three different cohorts. For assessing the validity of questions on midlife diet, retrospective intake of 56-72-year old individuals was estimated using the AGES-FFQ and results compared with detailed dietary data, gathered from the same individuals 18-19 years previously, i.e. in midlife, as a part of a national dietary survey. Validity of questions on current intake was assessed by comparing answers from healthy elderly individuals (≥65y) to data obtained from 3-day weighed food records completed by the same individuals. Participants of the AGES-Reykjavik Study, age 66-96 years (N=4798, 44% male), answered the complete AGES-FFQ, including questions on milk and cod liver oil intake in adolescence, midlife and current old age. BMD of femoral neck and trochanteric area was measured by volumetric quantitative computed tomography (QCT), and serum 25(OH)D concentration measured by means of a direct, competitive chemiluminescence immunoassay. A series of covariates from the AGES Reykjavik Study, as well as the Reykjavik Heart Study were also used in the analysis.

Results: The AGES-FFQ (on midlife and current diet) was found suitable to rank individuals according to intake of several important foods and food groups. Higher correlations were generally found for men than women. Questions on milk and cod liver oil consumption were among those with the highest correlation to the reference methods.

When assessing the association between milk intake at different periods of life and hip BMD in old age, the strongest association was found for midlife consumption, those with consumption of ≥once/day compared with <once/week having significantly higher hip BMD in old age. Women with current consumption of ≥once/day also had significantly higher BMD for trochanter compared with women consuming milk <once/week. Consumption in adolescence showed a similar trend, though insignificant. Associations were generally stronger for men than women.

Retrospective intake of cod liver oil in adolescence and midlife did not show a significant association with hip BMD in old age, neither did current intake for men. However, women with daily intake in current old age, had significantly higher BMD than those with intake of <once/week. Current cod liver oil intake was also associated with serum 25(OH)D concentration, showing increased concentrations with increased frequency of intake.

Conclusion: The AGES-FFQ can be used to rank individuals according to level of intake of several important foods and food groups, including cod liver oil and milk. When assessing the association between milk intake at different periods of life and hip BMD in old age, the strongest association was found for midlife milk intake, and stronger for men than women. Retrospective cod liver oil intake was not associated with hip BMD in old age, neither was current intake for men, while women with daily intakes had significantly higher hip BMD compared to those with intake of <once/week. Current intake was also positively associated with serum 25(OH)D.

Our results underline the importance of identifying critical dietary factors and time periods strongly associated with bone health in old age. Efficient preventive and health promoting measures may thus be developed, improving health, quality of life and independence in old age.

Keywords: Food frequency questionnaire, retrospective intake, current intake, validity, elderly, milk, cod liver oil, bone mineral density, 25(OH)D.

Ritgerð fyrir doktorsvörn í næringarfræði við Háskóla Íslands 2012

Ágrip

Inngangur: Fáar rannsóknir hafa beinst að gildi aðferða til að meta mataræði aldraðra, hvort heldur núverandi neyslu þeirra eða fæðuvenjur fyrr á lífsleiðinni. Sérstakur spurningalisti var unninn fyrir Öldrunarrannsókn Hjartaverndar með spurningum um mataræði á unglingsárum (14-19 ára), á miðjum aldri (40-50 ára) og núverandi mataræði aldraðra. Mikilvægt er að meta gildi listans til að koma í veg fyrir að rangar ályktanir verði dregnar um samband mataræðis og heilsufarsþátta.

Mjólk er mikilvægur kalkgjafi og lýsi veitir stóran hluta af D-vítamíni í fæðu Íslendinga. Í ráðleggingum um mataræði er mælt með reglulegri neyslu beggja fæðutegunda fyrir fólk á öllum aldri, ekki síst til að stuðla að heilbrigði beina. Hins vegar er ekki ljóst hversu sterkt samband er milli neyslu á mismunandi æviskeiðum og beinþéttni á efri árum, þegar mestar líkur eru á alvarlegum beinþynningarbrotum. Einnig er lítið vitað um samband næringar og beinþéttni meðal karla, þar sem fyrri rannsóknir hafa beinst að konum.

Markmið þessarar rannsóknar var að meta gildi spurninga um mataræði á miðjum aldri og núverandi mataræði. Niðurstöður um mjólkur- og lýsisneyslu á mismunandi æviskeiðum voru síðan nýttar til að kanna tengsl næringar við beinþéttni á efri árum meðal þátttakenda í Öldrunarrannsókninni.

Aðferðir: Gögn rannsóknarinnar eru fengin úr þremur mismunandi rannsóknarhópum. Við mat á gildi spurninga um mataræði á miðjum aldri var spurningalistinn lagður fyrir fyrrverandi þátttakendur úr Landskönnun á mataræði árið 1990, sem nú voru á aldrinum 56-72 ára. Niðurstöður listans voru síðan bornar saman við þá ítarlegu fæðissögu sem safnað hafði verið fyrir þessa sömu einstaklinga 18-19 árum fyrr, þ.e. þegar þeir voru á miðjum aldri. Við mat á gildi spurninga um núverandi mataræði var viðeigandi listi lagður fyrir hóp eldra fólks (65 ára og eldri), og niðurstöður bornar saman við þriggja daga matardagbók, þar sem sömu einstaklingar höfðu skráð og vigtað alla neyslu. Í rannsókn á beinþéttni svöruðu þátttakendur Öldrunarrannsóknarinnar öllum hlutum spurningalistans, auk þess sem beinþéttni þeirra var metin í vinstri mjöðm (nærenda lærleggs) með magnákvarðandi sneiðmyndatöku (QCT), og styrkur D-vítamíns (25(OH)D) í blóðvökva mældur.

Niðurstöður: Spurningalistinn var metinn hæfur til að raða einstaklingum eftir neyslu margra mikilvægra fæðutegunda. Fylgni milli aðferða var yfirleitt hærri meðal karla en kvenna. Spurningar um mjólkur- og lýsisneyslu voru meðal þeirra sem höfðu hvað hæsta fylgni við samanburðaraðferðir.

Við mat á sambandi mjólkurneyslu á mismunandi æviskeiðum við beinþéttni í mjöðm á efri árum, fundust sterkustu tengslin fyrir neyslu á miðjum aldri. Bæði karlar og konur sem neyttu mjólkur einu sinni á dag eða oftar á miðjum aldri voru með marktækt hærri beinþéttni en þau sem neyttu aldrei mjólkur eða sjaldnar en einu sinni í viku. Tengsl beinþéttni við núverandi neyslu voru aðeins marktæk meðal kvenna. Einnig fundust jákvæð tengsl milli mjólkurneyslu á unglingsárum og beinþéttni þótt þau væru ekki marktæk. Samband mjólkurneyslu og beinþéttni var yfirleitt sterkara meðal karla en kvenna.

Ekki fundust marktæk tengsl milli lýsisneyslu á unglingsárum eða miðjum aldri og beinþéttni í mjöðm á efri árum. Núverandi lýsisneysla tengdist hins vegar hærri beinþéttni hjá konum, en ekki hjá körlum. Samsvörun var milli lýsisneyslu aldraðra og styrks D-vítamíns í blóðvökva, þar sem bæði karlar og konur sem tóku lýsi einu sinni í viku eða oftar höfðu að meðaltali marktækt hærri styrk en þau sem tóku aldrei lýsi eða sjaldnar en einu sinni í viku.

Ályktun: Spurningalistinn var metinn hæfur til að raða einstaklingum eftir neyslu margra mikilvægra fæðutegunda, þar á meðal mjólkur- og lýsisneyslu. Samband mjólkurneyslu á lífsleiðinni og beinþéttni var jákvætt og sterkustu tengslin voru fyrir neyslu á miðjum aldri og sterkari meðal karla en kvenna. Tengslin milli lýsisneyslu og beinþéttni virðast minni, en aðeins sáust marktæk tengsl milli núverandi neyslu og beinþéttni meðal kvenna. Góð samsvörun var milli núverandi lýsisneyslu og D-vítamínstyrks í blóðvökva.

Mikilvægt er að kanna þátt mataræðis á mismunandi æviskeiðum fyrir beinheilsu aldraðra. Með auknum skilningi er hægt að þróa og efla forvarnir sem geta skilað sér í bættri heilsu og auknum lífsgæðum á efri árum.

Lykilorð: Tíðnispurningalisti um mataræði, mismunandi æviskeið, mjólkurneysla, lýsisneysla, beinþéttni aldraðra, D-vítamínstyrkur í blóði

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Abbreviations

AGES-FFQ	Food frequency questionnaire used and designed for the AGES-Reykjavik study
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
D	Day
DXA	Dual energy X-ray absorptiometry
FFQ	Food frequency questionnaire
H	Hour
INDS	Icelandic National Dietary Survey
IQR	Interquartile range
MMSE	Mini mental state examination
PA	Physical activity
PTH	Parathyroid hormone
QCT	Quantitative computerized tomography
SD	Standard deviation
W	Week
Y	Year
25(OH)D	25-hydroxyvitamin D, or calcidiol
1,25(OH)$_2$D	1,25-dihydroxyvitamin D, or calcitriol

List of original papers

This dissertation is based on the following papers, which will be referred to in the text by their respective Roman numerals:

I **Title**: Validity of retrospective diet history: assessing recall of midlife diet using food frequency questionnaire in later life.
Authors: Eysteinsdottir T, Gunnarsdottir I, Thorsdottir I, Harris T, Launer LJ, Gudnason V, Steingrimsdottir L.
Journal of Nutrition Health and Aging. 201:15(10):809-14.

II **Title:** Assessing validity of a short food frequency questionnaire on present dietary intake of elderly Icelanders.
Authors: Eysteinsdottir T, Thorsdottir I, Gunnarsdottir I, Steingrimsdottir L.
Nutrition Journal 2012:11(1):12.

III **Title:** Milk consumption throughout life is associated with bone mineral density in elderly men and women.
Authors: Eysteinsdottir T, Halldorsson TI, Thorsdottir I, Sigurdsson G, Sigurðsson S, Harris T, Gudnason V, Gunnarsdottir I, Steingrimsdottir L.
Manuscript

IV **Title:** Lifetime consumption of cod liver oil and bone mineral density in old age.
Authors: Eysteinsdottir T, Halldorsson TI, Thorsdottir I, Sigurdsson G, Sigurðsson S, Harris T, Gudnason V, Gunnarsdottir I Steingrimsdottir L.
Manuscript

1. Introduction

The aim of this dissertation is to increase knowledge on the significance of lifelong diet, particularly consumption of milk and cod liver oil, for bone health in old age. The Icelandic diet has traditionally been characterized by relatively high consumption of milk and dairy products and common use of cod liver oil. Data gathered in the Age/Gene Environment Susceptibility - Reykjavik (AGES-Reykjavik) Study provides a unique opportunity to assess the association between consumption of milk and cod liver oil at different periods of life and bone status in old age.

During the last century life expectancy has risen sharply in populations worldwide [1]. Consequently more people are reaching older age and the increase is not only in number but also as proportion of elderly individuals. The increase seen in the oldest age group, 80 years and older, is most striking, in Europe alone this group is expected to grow from 21.4 million individuals in year 2000 to 35.7 million by 2025 [2], and to 400 million worldwide by 2050 [3]. This development is likely to increase demand for both healthcare and social service systems, as the prevalence of chronic and degenerative diseases rises with increasing age [2,4].

One of the most prevalent diseases and major causes of disability in old age is osteoporosis and associated bone fractures. The number of hip fractures alone, the fractures that have the most impact on the individual and the highest medical cost, are expected to rise from 1.7 million in year 1990 to 6.3 million in 2050 [2], and worldwide cost of all osteoporosis fractures reaching 76.7 billion Euros in 2050 [5-7].

Various factors affect the risk of developing osteoporosis, such as age, gender, ethnicity and family history. These are beyond our reach, while others, including physical activity, smoking, alcohol consumption and last but not least dietary intake are largely under our control and can be modified to enhance the probability of healthy ageing.

While several dietary factors can affect bone health, the two most critical nutrients are considered to be calcium and vitamin D [8-10]. These nutrients are particularly important during periods of rapid growth in childhood and adolescence so that optimal peak bone mass may be achieved, and in old age to keep age-related bone loss to the minimum [11-14]. The main source of dietary calcium in Iceland is milk and dairy products, and cod liver oil is a traditional source of vitamin D [15-17].

There are few studies available on lifelong consumption of milk and cod liver oil, and the long-term association with bone health in old age is unclear. Furthermore, studies on elderly men are lacking in this area. A positive association between milk intake in childhood/adolescence and bone health in adulthood and even old age for women has been reported in both cross-sectional and cohort studies [18-24]. Despite being an excellent source of vitamin D, cod liver oil intake in childhood has been suggested to be adversely associated to bone health in old age [25].

More information is needed to better understand and assess the strength of the relationship between lifelong milk and cod liver oil intake and bone health in old age, when serious and debilitating osteoporotic fractures are most likely to occur.

2. Background

Various environmental factors can affect the development of chronic diseases and health in old age [1]. Eating habits and dietary intake play one of the key roles in this context [1,2,26], and are especially important as they can be modified to enhance, not only present health, but also the likelihood of healthy aging [26].

As life expectancy has increased considerably, the burden of various age related chronic diseases, including osteoporotic bone fractures, is expected to increase. Therefore it is more important than ever to identify factors that are strongly associated with bone mineral density (BMD) in old age, and develop efficient preventive and health promoting measures that are likely to improve health, quality of life and independence in old age [7,27].

2.1 Lifelong nutrition and health in late life

Studies have shown that nutrition in early life and throughout the lifespan can be related to development of chronic diseases and health in old age. Among associations seen are early life nutritional status and cognitive function in old age [28], restricted energy intake in childhood or early adulthood and reduced risk of ovarian cancer in elderly women [29], and milk intake in adolescence and increased risk of advanced prostate cancer among elderly men [30].

Bone mass in old age is determined by peak bone mass in early adulthood, and subsequent age related bone loss, both factors possibly affected by dietary intake and nutritional status [31]. The risk of suffering from osteoporosis and osteoporotic fractures in late life can thus be associated with nutritional adequacy from childhood and throughout the life span.

2.2 Osteoporosis

2.2.1 Definition

Osteoporosis is a chronic, progressive disease and has been defined by the World Health Organization (WHO) to be characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to bone fragility and a consequent increase in risk of fracture [32]. Osteoporosis can be classified into either primary or secondary osteoporosis. Primary osteoporosis is caused by age related bone loss in both genders and loss of gonadal function in men, while secondary osteoporosis is bone loss due to medical conditions, medications or nutritional deficits [33].

The following four diagnostic criteria can be used for assessing bone mass when measured with DXA. These criteria were set for postmenopausal white women [34], but can also be used for men age 50 years and older [34,35].

- Normal: BMD or bone mineral content (BMC) within 1standard deviation (SD) below the young adult reference mean.

- Osteopenia: Low bone mass, when BMD or BMC is between 1SD and 2.5SD below the young adult mean.
- Osteoporosis: BMD or BMC 2.5SD or more below the young adult mean.
- Severe osteoporosis: BMD or BMC 2.5SD or more below the young adult mean and at least one osteoporotic fracture.

2.2.2 Risk factors

Main risk factors for osteoporosis include age, gender, ethnicity, family history and previous fractures, as well as body weight, exercise, smoking, alcohol consumption, use of medications, and dietary intake [2,27,36,37]. The latter ones are of special interest as they are factors that can be modified to enhance the likelihood of healthy ageing.

In relation to dietary intake, the two nutrients most strongly associated to bone mass are calcium and vitamin D, and inadequate intakes have been linked to reduced bone mass and increased risk of osteoporosis [8,38,39,40].

While it is known that environmental factors, including nutrition, are related to the acquisition of bone mass during childhood, adolescence and early adulthood, the extent to which these factors can be modified to influence the risk of osteoporosis in old age is unclear [41].

2.2.3 Prevalence

Osteoporosis is the most common bone disease in humans, it affects both men and women and the prevalence increases with increased age [33]. Women are however affected earlier than men and are approximately three times more likely to get osteoporosis [27]. The higher prevalence seen among women is partly due to their lower peak bone mass as well as bone loss related to hormonal changes at menopause [27,42-46]. During menopause, estrogen production decreases, resulting in bone loss through increased rate of bone remodelling and calcium resorption from bone [42,43,45,46]. The fall in estrogen production can also decrease intestinal calcium absorption and increase urinary loss, causing even greater bone loss [44].

The risk of osteoporosis and osteoporotic fractures is different between different races; white women generally have lower BMD (less skeletal mass) than black women and higher fracture rates [47,48]. For white women, approximately 40% of 70-79 year olds, and 70% of women 80 years and older are expected to suffer from osteoporosis [34]. Furthermore, there also appears to be a difference within races, and white women of Northern Europe, especially Scandinavian, tend to have particularly high fracture rates [49-51].

Even though osteoporosis is often considered a women's disease, it is increasingly recognized as a significant cause of morbidity and mortality in men, with high numbers of affected men. Data from the 3rd National Health and Nutrition Examination Survey (NHANES III) indicate that 3-6% (1-2 million) of men 50 years old or older in the US suffer from osteoporosis and 28-47% (8-13 million) have osteopenia [52].

Adult bones have two types of tissue, cortical outer bone, which is more compact and is remodeled less frequently than the inner, spongy trabecular bone [27,53]. With advancing age, men are generally thought to lose 15–45% of trabecular bone and 5–15% of cortical bone, whereas women lose 35–50% of trabecular bone and 25–30% of cortical bone [34].

As early bone loss and osteoporosis is generally asymptomatic, cases are often not diagnosed until after a fracture occurs [27] and prevalence is more likely to be underestimated than overestimated.

2.2.4 Fractures

The morbidity of osteoporosis is generally not caused by the bone loss itself, but the fractures sustained due to the lower bone mass and microarchitectural deterioration of the bone tissue [27, 34]. Osteoporotic fractures occur after low trauma, e.g. falling from standing height or less, or even after coughing, sneezing, or sudden movements [33]. These fractures are most common in the wrist, spine and hip, and increase exponentially with age – especially hip fractures, which are also the fractures that have the most impact on the individual and the highest medical cost [34]. Women have higher lifetime risk of osteoporotic fractures [54], and an increase in fracture incidence is seen at younger age for women than men [34,55]. Nevertheless, osteoporotic fractures also cause substantial morbidity among men, and men tend to have worse outcomes after fractures, including hip fractures, where men have higher excess annual mortality than women, comparing age- and sex-matched individuals with and without fractures [56,57]. The number of hip fractures alone are expected to rise from 1.7 million in 1990 to 6.3 million in 2050 (WHO 2002), with worldwide cost of all osteoporosis fractures reaching 76.7 billion Euros [5-7].

2.2.5 Prevention

Considering preventive measures, main focus has been on the role of lifestyle factors such as diet, exercise, avoidance of smoking, and limited alcohol consumption, as well as hormonal status, use of medications, and maintenance of healthy body weight [27,34,37,58]. It is however possible that the importance of different lifestyle factors may vary between life stages [27], and it is therefore important to assess them separately.

In relation to diet, calcium and vitamin D have been shown to be particularly important when it comes to osteoporosis prevention [27]. The risk of osteoporosis in old age is strongly influenced by peak bone mass, which is partly determined by adequate calcium and vitamin D intake in childhood and adolescence [31]. Sufficient intake from childhood and throughout life is therefore likely to be important when it comes to maintaining bone mass and lowering the risk of osteoporosis and osteoporosis related fractures in old age. Calcium and vitamin D supplementation among elderly individuals may also lower fracture risk, especially in previously deficient individuals [59-62].

While preventive measures for those with low absolute fracture risk generally focus on lifestyle factors, those with higher fracture risk may also require pharmacological interventions [27]. The availability of medications that effectively reduce the risk of osteoporotic fractures has increased in recent years. Clinical trials have shown certain oral bisphosphonates to be able to decrease the risk of vertebral and hip fractures by 50-60% in postmenopausal women [63-65], as well as reducing fracture rates in men with osteoporosis [66].

2.3 Calcium and vitamin D, roles in the body and dietary sources

2.3.1 Calcium, vitamin D and bone

Calcium is the most prevalent mineral in the body and 99% of it is stored in bone and teeth [67]. Calcium deposition into the skeleton is critical for its structure and is necessary for tissue rigidity, strength, and elasticity. The skeleton also functions as a source of minerals, like calcium, and is critical for homeostasis. Serum calcium concentration is tightly regulated by parathyroid hormone (PTH), calcitriol (1,25 dihydroxyvitamin D, the active form of vitamin D), and calcitonin [68,69]. It is important to maintain optimal calcium levels, as they are needed for mineralisation of bone, muscle contraction, nerve conduction and many other cellular functions [53]. If calcium concentration lowers slightly the parathyroid glands secrete PTH, which activates bone resorption and stimulates the production of calcitriol. Calcitriol in turn increases intestinal calcium absorption, and together with PTH stimulates bone resorption [70]. Thirdly, calcitriol and PTH act on the renal distal tubule to reabsorb calcium for maintaining normal calcium levels [71]. PTH secretions then decreases as calcium levels increase [53].

Adequate intake of calcium is critical for normal growth and achieving optimal peak bone mass as well as for modifying the rate of age-related bone loss [12-14]. The main source of dietary calcium in Iceland is milk and dairy products [15-17].

Inadequate intake of calcium has been linked to reduced bone mass and increased risk of osteoporosis [8]. If vitamin D is also insufficient, this can further increase fracture risk [8,38-40], as active calcium absorption is then reduced and secretion of PTH increases, which can lead to increases bone turnover and bone loss [39,59,61]. If however vitamin D levels are adequate, it is possible to achieve calcium absorption at lower intake levels, compared to when vitamin D levels are insufficient [72].

Chronic vitamin D deficiency is known to cause rickets among children and osteomalacia among adults [53].

2.3.2 Milk

Consumption of milk and dairy products is a good indicator of calcium intake in Iceland, both during present and earlier times. Traditionally, consumption of milk and dairy products has been high in Iceland, although there has been a decrease in consumption over the last decades. In 1990 the average consumption of milk and dairy was approximately 600ml/d, not including cheese, in 2002 it was around 400ml/d, and 300ml/d in 2011 [15-17]. The consumption of cheese was relatively low in earlier times, but has been rather stable over the past two decades, with average consumption of approximately 35-40g/d. Despite the decrease in milk and dairy consumption, average consumption is still quite high and adult Icelanders receive 65% of their dietary calcium from milk, dairy products and cheese [17]. In previous Icelandic

National Dietary Surveys (INDS) the percentage used to be even higher or approximately 70% [15,16].

Other sources of calcium have been limited, such as calcium depleted water supply (4.8mg/L), and minimal consumption of green vegetable or calcium rich small fish, such as sardines [15-17]. Furthermore, calcium fortification of foods has not been common, and was virtually non-existent for the most part of the 20th century. Therefore it can be assumed that individuals consuming little or no milk or dairy products most likely had low calcium intakes.

Reflecting the high milk intake in Iceland, average calcium intake has in previous INDSs been quite high and above the recommended 800mg daily intake (RDI) for adults of both genders and in all age groups [15,16]. According to INDSs average calcium intake was approximately 1300mg/d in 1990 and 1100mg/d in 2002 [15,16]. In the most recent INDS average intake was slightly over 900mg/d and was above RDI for all groups except the oldest group of women, 60-80y, which had an average intake of approximately 700mg/d [17].

Milk and dairy products are nutrient dense foods, not only supplying calcium, but also providing other nutrients and factors that may promote bone growth and calcium accretion, such as proteins, peptides, phosphates, potassium and magnesium, as well as growth factors and other hormones [73,74]. It is also probable that milk contains other components yet to be identified that affect bone density. It should be noted that milk is generally not fortified with vitamin D in Iceland.

2.3.3 Cod liver oil

Vitamin D can be synthesized in the skin when it is exposed to sunlight of the appropriate wavelength. However, due to the latitude of our country, reaching from 64-66°N, dermal production of vitamin D is limited approximately from October to April [75]. Intake of vitamin D containing supplements is therefore recommended, and cod liver oil is the one most traditionally used in Iceland.

Cod liver oil is rich in vitamin D, vitamin A (in the form of retinol), and essential omega-3 fatty acids. Around the mid 20th century there was limited control over the amount of vitamin A and D in cod liver oil and the ratio between the vitamins probably reflected that of the pure cod liver (83:1) [76]. In later years and up until 2002, cod liver oil contained approximately 30.000:250µg/100g of vitamins A and D respectively (equalling 120:1). Subsequently the concentrations of vitamin A were lowered, and today the amounts are 5.000µg and 200µg/100g of vitamin A and D respectively. Before this change the recommended spoonful per day (approximately 5g) of cod liver oil contained 1650µg of vitamin A and 12.5µg of vitamin D, but now contains 250µg and 10µg of vitamin A and D respectively. Recommended daily intake (RDI) of vitamin A is 700µg for women and 900µg for men and 10µg of vitamin D for adults and 15µg for elderly individuals [77].

Some epidemiological studies have linked high intake of vitamin A/retinol to adverse effects on bone health and increased risk of osteoporosis and osteoporotic fractures [78-82], particularly if serum (25(OH)D) is also low [83].

Cod liver oil is a traditional source of vitamin-D in other Nordic countries as well and regular consumption is recommended. The cod liver oil in commercial use in Norway also contained high concentration of vitamin A in earlier timers but the concentration was decreased from 20,000µg/100ml to 5,000µg/100ml, same as in Iceland [25]. A follow-up study of 50-70 year-old women in the Norwegian Nord-Trøndelag Health Study, found an association between childhood consumption of cod liver oil and current forearm BMD, where women reporting any intake in childhood had significantly lower BMD then those with no intake [25]. The association appeared to be a negative dose-response as they found an increasing odds ratio for low BMD with increasing regularity of childhood cod liver intake. The researchers concluded that the previous high concentration of vitamin A in cod liver oil, when added to an already vitamin A rich diet, may have lead to total intake reaching harmful levels.

However, as studies are few, it is still not clear whether vitamin A from cod liver oil, with its high vitamin D content, has adverse effects on bone health. In a British case-control study of women over 75 years of age, Barker et al [84] found that intake of cod liver oil or multi-supplements (most multi-supplements in UK contain retinol) was associated with lower risk of any fracture. Sigurdsson et al [85] also found no adverse association between intake of preformed retinol (mostly derived from cod liver oil and multivitamins) and BMD at different skeletal sites in a cross-sectional study of 70 year old women in Iceland. Neither of these two studies involved intake in adolescence or at young age, however, and it is possible that the growing bone may be differently affected than adult bone.

2.3.4 Assessing vitamin D status; 25-hydroxyvitamin D (25(OH)D), association with dietary vitamin D and optimal levels

Calcitriol, or 1,25-dihydroxyvitamin D, is the active form of vitamin D, however, it is not considered a good indicator of vitamin D status as its half-life is relatively short and concentrations are tightly regulated [86]. There is however a general consensus that serum 25-hydroxyvitamin D (25(OH)D), or calcidiol, is currently the best available indicator when it comes to assessing vitamin D status, as it is well suited to reflect total amount of vitamin D, both that ingested as a part of the diet (including supplements), as well as that from dermal production [87].

According to the Institute of Medicine [53] data suggest that individuals are at risk of vitamin D deficiency when serum levels of 25(OH)D are below 30nmol/L, and some may be at risk for inadequacy at levels between 30 and 50nmol/L. Serum levels of ≥50nmol/L should be sufficient for majority of people.

Serum 25(OH)D increases in response to increased intake of vitamin D, and according to a meta-regression analysis an increase of 1-2nmol/L was found for each additional 100IU (2.5µg) of vitamin D [88,89]. However, dose-response relationship can differ as this relationship seems to be affected by several factors, such as baseline value of 25(OH)D and duration of supplementation in supplemental studies [67,90-92], e.g., it requires higher amounts of vitamin D to increase a 25(OH)D serum level which is already above 50nmol/L, compared to levels below 50nmol/L [90]. Furthermore, supplementation studies have shown that the rise in 25(OH)D levels for a given dose tends to stabilize with time [91,92].

2.4 Intake of milk/calcium and cod liver oil/vitamin D during different periods of life and association with bone health in old age

2.4.1 Early life

Dietary requirements for calcium vary throughout the life stages, as they are determined by the needs for bone development and maintenance, with greater needs e.g. during rapid growth in childhood and adolescence, and in old age to keep age-related bone loss to the minimum [11-14]. As bone mass in old age relies partly on peak bone mass achieved in early adulthood, good bone health in old age is to some extent dependent on adequate calcium intakes during childhood and adolescence [12,74].

Although intervention studies have shown that calcium supplementation in childhood and adolescence can modestly increase bone mass, this increase is generally found to be transient [93-95] and is likely to benefit mainly children with low habitual intakes of calcium [96]. Conversely, both cross-sectional and cohort studies have reported a positive association between milk intake in childhood and/or adolescence and bone health in adulthood and even old age for women [18-24].

In adolescence adequate vitamin D is important as bone accretion can be extensive due to rapid growth. This is reflected in the increased conversion of 25(OH)D to calcitriol, the active form of vitamin D, at the start of puberty [53]. Even after growth ceases, our bones constantly undergo remodelling and adequate vitamin D is therefore important at all ages.

It is well known that vitamin D deficiency can cause rickets in children [67], but it is also possible that marginal deficiency during early life may contribute to osteoporosis risk in later life [87].

Intake of a vitamin D containing supplement is recommended in Iceland for people of all ages, and cod liver oil is traditionally used. Cod liver oil was even given to children in most schools in the 1930s and up until around 1970. Although cod liver oil is largely recommended for bone health, the association between intake of cod liver oil in childhood and bone health in old age is not well studied. It has even been suggested that childhood consumption in earlier decades might even be associated with adverse affect on bone health in current old age, as cod liver oil used to have extremely high levels of vitamin A [25]. More studies are needed to clarify this relationship.

2.4.2 Adulthood/midlife

Throughout most of our adulthood bone formation and resorption is balanced and calcium requirements are stable [53]. Consequently bone mass is relatively stable until the onset of menopause in women, when hormonal changes result in rapid bone loss. Men also start to lose bone minerals from all skeletal sites when age-related bone loss sets in, at approximately 50 years of age. This loss is however more gradual than in women [97,98].

Some studies have suggested calcium interventions to be more effective during the late postmenopausal period compared to the peri-menopausal period in women [99]. Others have nevertheless found a positive association between milk intake in early adulthood and midlife and bone health among elderly women [19-21]. As an example, in a cohort study by Soroko et al [21] a graded significant association was found between midlife milk consumption and BMD of elderly women. Murphy et al [20] also reported an insignificant, upward trend in BMD in elderly women, associated with increased milk consumption during adulthood and middle age.

As our bones are constantly undergoing remodelling, vitamin D is important at any age, and middle age is no exception. There is however a lack of studies on intake of vitamin D and cod liver oil in midlife and possible association to bone health in old age.

2.4.3 Old age

In old age dietary calcium intake generally decreases, while calcium requirements increase with increasing age, partly because of decreased intestinal absorption [12,33]. This may result in reduced supplies of calcium which again can be associated with decreased bone mass and increased risk of osteoporosis [8]. Controlled trials have shown that calcium supplementation in elderly women can reduce age-related bone loss [100,101]. Additionally, milk supplementation has been found to diminish bone turnover among postmenopausal women [102].

Adequate intake of both calcium and vitamin D is important for preservation of bone mass and prevention of osteoporosis [8], and for those who already suffer from osteoporosis, vitamin D insufficiency may amplify bone loss and further enhance fracture risk [8]. In old age we rely even more on dietary vitamin D as dermal production of vitamin D is reduced in old age [33].

Intervention studies on vitamin D (often also including calcium supplements) have found an association with increased BMD of the femoral neck [103] as well as decrease bone loss [104-106], fall risk [107] and risk of osteoporotic fractures [62,104-106,108] among elderly individuals. Still it is unclear how much vitamin D is needed for beneficial effects.

2.5 Assessment of dietary intake methods

In order to study the association between diet and various health related factors, we need high quality health data and valid methods for measuring dietary intake. Food intake can be assessed on three different levels; national, household and individual level. Data collected on an individual level is the only data that can provide information on average intake of various foods and nutrients and their distribution in well defined groups of individuals [109,110]. Such data can also be used to estimate adequacy of dietary intake and to assess the relationship between diet and health [111,112]. Several different methods can be used to collect data on an individual level and each method has its strength and limitations. Data can be collected either retrospectively or prospectively and the methods most commonly used are twenty-four-hour recall, diet history, food frequency questionnaires, and food records. When choosing an appropriate method for a study, several factors have to be taken into account, such as aims of the study, characteristics of the study group/population, and practical issues such as various resources (funding, man power etc). Assessing dietary intake is always a challenge, and perhaps even more so when assessing intake among the elderly. Various factors related to older age, such as declined cognitive function, fading memory, impaired hearing and/or vision, may possibly affect the ability to give reliable information on dietary intake [113-117].

2.5.1 Food records

When this method is used, either the respondent, or an observer, records in detail all food and beverages consumed over a specified period of time, usually including brand names and meal preparation methods. Foods can be either weighed accurately using a household scale, or estimated using household measurements such as glasses, bowls and spoons [110,118,119]. As recording is done at every mealtime, this method does not rely on the recorder's memory. A food record of 3-4 consecutive days, and usually no more than 7 days, is generally recommended, as studies have shown that incomplete records get more frequent as the number of days increases, this has been referred to as respondent fatigue [110,112,120]. If more days are needed, repeated recordings can be collected. When it comes to assessing dietary intake, weighed food records have often been considered as the "gold standard" as they can provide relatively accurate quantitative information on consumption [110,118,119]. However, this method has rather high participation burden, and the recording can also alter usual dietary behaviour and affect both the types of food and amounts being consumed [110,118,119]. Processing the data is also expensive and time consuming [118].

Elderly participants have proved to be capable of keeping food records with acceptable levels of compliance and completion [121], and food records have been found to provide valid intake data for free-living elderly individuals [122].

2.5.2 24-hour recall

In the 24-hour recall interview the respondent is asked to remember and report all food and beverage consumed during the previous 24 hours or the previous day [110,118]. As day to day variability can be great, data from single 24-hour recalls should not be used to estimate usual diets of individuals or prevalence of high or low intakes, for that purpose repeated recalls are needed [110,112,119]. Single 24-hour recalls can however be used to assess average intake of populations, and are commonly used in national nutrition surveys [16,17,124,125]. By using the 24-hour recall, a relatively low burden is placed on the respondent, and the method does not affect the dietary choices of the participants. The major limitation of this method however, is that it relies on the respondent's memory, both regarding food items and portion sizes.

2.5.3 Diet history

The diet history usually involves a combination of methods, including a detailed interview, and a food frequency questionnaire, incorporating frequency as well as quantity of foods [110,126,127]. This method can provide detailed information on food intake, along with usual meal patterns, eating habits, preparation of meals and meal composition [118,119]. Carrying out this method requires skilled staff, and is both time consuming and expensive, with a relatively high respondent burden. The diet history also partly relies on memory. Since the diet history often consists of rather long interviews and questionnaires, it may not be the most appropriate method to use for older individuals [116].

2.5.4 Food frequency questionnaire

A food frequency questionnaire (FFQ) is essentially a list of food items and suitable response categories, where the respondent is asked about frequency of consumption of listed items [110,112,119,128]. FFQs can be limited to frequency of consumption, or may also include estimated portion sizes, in which case they are termed semi-quantitative FFQs [110,118]. The structure of an FFQ is crucial, number of questions and responses need to be appropriate, and they should be designed for, or adapted to, the intended study population [110]. FFQs are widely used in epidemiological studies as they are easily completed and both data collecting and processing are relatively inexpensive. The main limitation of this method is that it relies on the respondents' memory. Also, many details are not measured and little information is generally collected on cooking and combination of meals [118]. While short FFQs lack the detail of longer questionnaires or food records, they have nevertheless been found to adequately assess the intake of specific foods and rank individuals according to level of consumption [129-132].

It has been suggested that FFQs may be a more appropriate dietary assessment method for older people than, for example, 24 hour recalls [116,133] as older individuals may have more problems with short-term than long-term recalls, as well as more difficulties with open-ended recalls than with structured ones [117]. The length of interviews and questionnaires is also crucial as older people may require longer time to answer and may become more fatigued and frustrated than younger people [116]. Long and extensive FFQs may contribute to lower response rates among elderly people [115] and shorter FFQs may therefore be more appropriate.

Food frequency questionnaires have become key research tools in nutritional epidemiology and assessing their validity is essential, as incorrect information may lead to false associations between dietary factors and diseases [134].

3. Aims

The aim of this dissertation was to increase knowledge on the significance of lifelong diet, particularly consumption of milk and cod liver oil, for bone health in old age.

Specific aims were to:

- Assess the validity and ability of the AGES-FFQ on midlife diet to rank individuals according to intake of selected foods and food groups, and to distinguish between individuals with high versus low intake.

- Assess the validity and ability of the AGES-FFQ on intake in current old age to rank individuals according to intake of selected foods and food groups, and to distinguish between individuals with high versus low intake.

- Investigate whether, and to what extent, retrospective self-reports on milk consumption in adolescence and midlife, as well as current consumption, is associated with hip BMD in elderly men and women of the AGES-Reykjavik Study.

- Assess the association between retrospective self-reports on cod liver oil intake in adolescence and midlife and hip BMD in old age.

- Assess the association between current cod liver oil intake and hip BMD in old age.

- Assess the association between current intake of cod liver oil and serum 25(OH)D concentration in old age.

4. Methods

4.1 Study design

The first part of this thesis is on validity of a food frequency questionnaire designed and used in the AGES-Reykjavik Study, the AGES-FFQ. The AGES-FFQ includes questions on frequency of consumption at three different periods of life; adolescence, midlife and in current old age. Validity of questions on midlife and current intake is assessed here. In the second part of the thesis, association between milk and cod liver oil consumption at different periods of life and hip BMD in current old age is assessed. Also, the association between current cod liver oil intake and serum 25(OH)D concentration was investigated.

4.2 The AGES-Reykjavik Study

The AGES-Reykjavik Study is conducted by the Icelandic Heart Association and the National Institute on Aging Intramural Research Program. AGES-Reykjavik originates from the Reykjavik Study, a large population-based cohort study initiated in 1967. All men and women born in 1907-1935 (n=30,795) and residing in Reykjavik and nearby communities in 1967 were selected, equalling roughly 35% of this age-specific population in Iceland at that time. Thereof 27,281 were invited to participate and 19,381 attended (71%) [135-138]. Of the 11,549 cohort members still alive in 2002, 8,030 individuals were randomly chosen and invited to participate in the AGES–Reykjavik Study. At the end of the enrolment period in 2006, 5,764 individuals (66-96y; 42% male) had been examined.

The AGES–Reykjavik study was designed to examine risk factors, such as genetic susceptibility and gene/environment interaction, including diet, in relation to disease and disability in old age. Extensive data were collected during clinical examinations, e.g. on physical and cognitive function, anthropometry, health history, and food history during adolescence, midlife and current old age. Participants also underwent quantitative computerized tomography (QCT) scanning and were asked to bring to the clinic all medications and supplements used in the previous two weeks, representing present usage [136,139]. The AGES–Reykjavik examination was completed in three clinic visits within a 4- to 6-week time window.

4.3 Study population

This thesis consists of data from three different study cohorts: 1) A subsample of former participants of the 1990 Icelandic National Dietary Survey (INDS), 2) A subsample of elderly individuals taking part in another geriatric study, the IceProQualita Study, 3) Participants of the AGES-Reykjavik Study.

4.3.1 Cohort 1 – Validation of retrospective food frequency questions

In 1990 a large national dietary survey was carried out in Iceland (1990 INDS). The sample for that survey included 1725 individuals, aged 15-80, selected randomly from the national registry of that time. Detailed dietary history was gathered and participation rate was 72%. Characteristics of that study population have been described elsewhere [15]. As the aim of the first study (paper I) was to assess validity of retrospective questions on dietary intake in midlife (40-50y), individuals who were middle aged at the time of the 1990 INDS were invited to participate in the validation study. A total of 305 individuals, born 1937-1952, were in the original sample and were contacted in 2008-2009 and sent the AGES-FFQ on midlife diet. Of these, 174 returned completed questionnaires (57%), 107 women and 67 men. Their average age at the time of the 1990 survey was 44 years. Results from the AGES-FFQ were then compared to the detailed dietary data gathered in the 1990 INDS.

4.3.2 Cohort 2 – Validation of food frequency questions regarding consumption in current old age

Subjects in Cohort 2 were healthy, elderly people, 65 years and older (58.6% female), and were a subsample of participants of the IceProQualita study. Participants were recruited into the IceProQualita by advertisements posted in community centres and residential care homes in the capital area of Iceland. Exclusion criteria for the study were cognitive function <19 points on the Mini Mental State Examination (MMSE) [140], uncontrolled coronary heart disease, pharmacological interventions with exogenous testosterone or other drugs known to influence muscle mass, and major orthopaedic disease. Participant also had to be free of any musculoskeletal disorders, had to be weight stable and all women postmenopausal. Details of the IcePeoQualita study have been described elsewhere [141-143].

Our subsample consisted of the first 137 participants enrolled into the IceProQualita Study by March 2009, when data analysis for the present study began. Data from nine participants were not considered adequate; hence data from 128 individuals was included. Average age of participants was 74 years. Our subsample did not differ from the whole study group of the IceProQualita study regarding age, anthropometric measurements, physical performance test, and outcome of various questionnaires on, e.g., general health, quality of life, and the MMSE. Results from the AGES-FFQ on intake in current old age were compared to dietary data obtained from 3-day weighed food records completed by the same individuals.

4.3.3 Cohort 3 – Association between lifelong consumption of milk and cod liver oil and hip BMD in old age.

Characteristics of participants of the AGES-Reykjavik Study have been described in detail elsewhere [135,136]. Of the 5,764 participants of the AGES-Reykjavik Study, 933 individuals did not undergo the QCT scanning and additionally 33 individuals did not give adequate dietary information. Therefore data from 4,798 individuals, average age 76 years (44% male), were used when assessing the relationship between lifelong consumption of milk and cod liver oil and BMD in old age in the present studies.

4. 4 Data collection

4.4.1 The diet history – paper I

In the 1990 INDS dietary information was gathered using detailed dietary history, focusing on the last three months. Each participant met with an interviewer in an hour-long interview taken in the participant's home, work place or a local clinic. The interviewers (n=32) were teachers or students of nutrition or food sciences. They took a ten-day course prior to the survey for training and synchronizing methods. When assessing usual diet, the interviewer asked about each meal/snack of the day, which food groups/items were usually consumed, how often and in what quantities, also which cooking methods were commonly used (baked/boiled/fried, etc.). Quantities were estimated using photographs of different portion sizes, as well as measurement glasses and bowls. Consumption was recorded as times per month, and daily intake of food (grams per day) calculated accordingly. The 1990 INDS has been described in detail in Steingrimsdottir et al. [15].

4.4.2 The 3-day weighed food record – paper II

In the IceProQualita Study each participant met with a researcher at baseline, and was provided with a household scale (PHILIPS Essence HR 2393) and a structured booklet for recording his or her intake for three consecutive days (Thursday-Saturday or Sunday-Tuesday). Participants received detailed oral instructions on how to weigh and record their intake and were shown how to use the household scale. Written instructions were also incorporated in the booklets, along with contact information, in case any questions arose during recording. The importance of maintaining their regular diets and weighing and recording all food and drink consumed was emphasized.

The number of recorded days was limited to three, as longer recordings were considered too demanding for the elderly participants.

4.4.3 The food frequency questionnaire

The food frequency questionnaire used in the present studies was developed especially for the participants of the AGES-Reykjavik Study. The questionnaire is divided into three parts, asking about intake at different periods of life; 16 questions on adolescent intake (14-19y), 17 questions on midlife intake (40-50y) and 30 questions on intake in current old age. The questions are on average frequency of intake of major food groups, e.g., milk and dairy products, meat, fish, bread, fruits and vegetables, and on selected foods, such as rye bread, blood/liver sausage, oat meal porridge and cod liver oil. Foods and food groups were selected for the questionnaire on basis of their contribution to absolute intake of elderly Icelanders according to National Nutrition Surveys [15], as well as their unique nutritional qualities and suspected connection to the development of various diseases in later life.

Majority of the questions have the same possible response categories, ranging from "Never" to "More than once a day" (Figure 1), as the questionnaire was designed to be simple and easily completed by elderly individuals. Questions on coffee, tea and sugar in coffee/tea differ in that way that they ask about daily frequency rather than weekly frequency of consumption. Additionally, questions related to types of products, such as low fat vs. high fat, and salt perception also have different response categories; the validity of these questions was not assessed in the present studies.

The AGES-FFQ was used to assess frequency of consumption of different foods and food groups in order to rank individuals according to their level of intake.

Subjects in Cohort 1 answered the part of the AGE-FFQ on midlife diet, subjects in Cohort 2 answered the part of the AGES-FFQ on present diet and subjects of Cohort 3, the participants of the AGES-Reykjavik Study, answered the complete AGES-FFQ.

Figure 1. Example question from the AGES-FFQ

On average, how often did you drink milk or eat dairy products when you were middle aged (40-50y)?

- ☐ Never
- ☐ Less than once a week
- ☐ 1-2 times a week
- ☐ 3-4 times a week
- ☐ 5-6 times a week
- ☐ Daily
- ☐ More than once a day

4.4.4 Bone variables – papers III and IV

QCT measurements, providing true volumetric density, were performed on the left hip using a 4-detector CT system (Sensation, Siemens Medical Systems, Erlangen Germany). Scans were acquired using a standardized protocol and encompassed the proximal femur from a level 1cm above the acetabulum to a level 5mm inferior to the lesser trochanter with 1mm slice thickness. Further procedures and quality assessments have been described in detail elsewhere [136,144].

The variables used in the present study are volumetric integral BMD (g/cc), reflecting both trabecular and cortical bone mass, of both femoral neck and trochanteric area, encompassing both trochanters. Individuals who were not able to lie supine, were over 150kg, or had undergone hip replacement surgery, were excluded from the QCT hip scans.

BMD of the hip was chosen over other skeletal sites due to the great impact hip fractures generally have on the elderly, their quality of life and life expectancy, as well as their substantial cost for the health care systems [2].

4.4.5 Measurements of serum 25-hydroxyvitamin D – paper IV

Measurement of 25(OH)D was conducted by means of a direct, competitive chemiluminescence immunoassay using the DiaSorin LIAISON 25(OH)D TOTAL assay (DiaSorin, Inc., Stillwater, Minnesota).

4.4.6 Other data collected in the AGES-Reykjavik Study – papers III and IV

Extensive data were collected in the AGES-Reykjavik Study during clinical examinations, e.g. on physical and cognitive function, physical activity at different periods of life, anthropometry, alcohol consumption, and smoking habits. Participants were also asked to bring to the clinic all medications and supplements used in the previous two weeks, representing current usage [136,139].

To obtain history of physical activity, participants were asked how much time (hours/week) they spent on moderate to vigorous activities in four different periods of life; young adulthood (20-34y), early middle age (35-49y), late middle age (50-64y) and current physical activity. Both weight bearing and non-weight bearing exercises were included. Consumption of alcohol was measured by asking about current consumption, converted into grams per week using 14g of alcohol as a standard drink.

Data on midlife BMI, which had been collected in the Reykjavik-Study [135], was also used.

4.5 Data management and statistical analysis

4.5.1 Data management

4.5.1.1 Paper I

To assess correlation between the 1990 dietary data and the answers from the AGES-FFQ on midlife diet from Cohort 1, the 1990 data was transformed into times per week, agreeing with the classification of the answers from the AGES-FFQ. Predetermined portion sizes were used, taking into consideration both average daily consumption from the 1990 INDS and recommended portion sizes from the Public Health Institute of Iceland (Table 1). The part of the questionnaire on midlife diet contains seventeen questions, eleven of which were assessed here. The remaining six questions were either not directly on frequency of intake (type of milk, type and amount of bread spread commonly used) or intake was too low or sporadic according to the AGES-FFQ to be compared to the 1990 INDS data (fish toppings, salted/smoked meat/fish).

For the cross-classification, data was split into five groups with graded level of intake, and the proportions of subjects falling into the same groups by the two methods was assessed. Since distribution of reported intake according to the AGES-FFQ was skewed, subjects could not be divided into quartiles or quintiles. For that reason the two lowest possible answers of the AGES-FFQ were combined, as well as the two highest ones. That left 5 levels of intake: level 1 – lowest intake, never or less than once a week; level 2 - equalling 1-2 times per week; level 3 - equalling 3-4 times per week; level 4 - equalling 5-6 times per week and level 5 being the highest level - equalling daily intake or more than once per day. Data from the 1990 INDS was also transformed into categories agreeing with the 1-5 classification of the AGES-FFQ answers.

Table 1. Portion sizes used in paper I.

Food/food group	g/portion
Meat meal	150
Fish meal	150
Potatoes	120
Fruits	100
Blood-/liver sausage	70
Rye bread/flatbread	40
Whole-wheat bread (2 slices)	54
Oatmeal/muesli	100
Vegetables	100
Milk/dairy products (1 portion each)	400
Cod liver oil (1 tablespoon)	13

4.5.1.2 Paper II

The part of the questionnaire on present diet includes 30 questions, 21 of which were assessed here. The remaining nine questions, not assessed in the present study, are on the frequency of hot meals, type of milk and dairy products most commonly used, type and amount of bread spread commonly used, and finally there are four questions related to salt consumption (perception of saltiness, consumption of salted meat, salt fish, and added salt to prepared meals).

Data on the participants' intake according to the 3-day weighed food records (gathered from Cohort 2) were entered into an interview-based nutrient calculation program, ICEFOOD, designed for the national dietary survey of The Icelandic Nutrition Council [16]. Individual intake in grams per day for each food/food group was calculated from 452 food recipes, which are based on 1148 food items from the National Nutritional Database, ISGEM.

Gender-specific portions were estimated taking into account actual intake in grams and eating occasions from the food diaries, as well as predetermined portion sizes used in our previous validation study on questions on midlife diet (Table 2). The gender-specific portions were then used to calculate intake in grams from the AGES-FFQ. Correlation was then calculated between grams of food intake according to the two methods, the 3-day food record and the AGES-FFQ.

Table 2. Portion sizes used in paper II.

Food/food group	Men g/portion	Women g/portion
Meat	200	135
Fish	170	130
Fish toppings	45	70
Potatoes	110	85
Fresh fruit	110	120
Blood/liver sausage	80	60
Rye bread/flatbread	50	60
Whole wheat bread	50	45
Oatmeal/muesli	190	150
Cocked vegetables	90	90
Fresh vegetables	90	80
Cakes and cookies	70	60
Candy	35	30
Dairy products	205	170
Milk	165	135
Pure fruit juice	160	160
Soft drink and sweet juice	345	225
Fish liver oil	5.6	7.2
Coffee	210	195
Tea	220	240
Sugar in coffee/tea	5	5

4.5.1.3 Papers III and IV

Milk and cod liver oil consumption was categorized *a priori* into three groups: ≤ once/week, 1-6 times/week, and daily (cod liver oil) or ≥once/day (milk).

Due to the approximate normal distribution of the source BMD variables in our population they were transformed into sex-specific z-scores, reflecting the number of standard deviations (SD) from age-related mean BMD.

4.5.2 Statistical analysis

4.5.2.1 Papers I & II

Statistical analysis was done using the computer program SPSS (version 11.0; Chicago, IL, USA). Kolmogorov-Smirnov tests were used to assess normal distribution of data. Simple descriptive statistics were used to describe general characteristics of the study groups. To assess differences between groups, student t-tests, Mann-Whitney U-test and Chi-square test were used. Spearman's rank correlation test was used to assess correlation between answers from the AGES-FFQ and reference methods. To further assess association between dietary methods, cross-classification was used in paper I, assessing proportion of individuals falling into the same, adjacent or opposite category according to intake in the two methods. In paper

II data from the AGES-FFQ was however split into 2-4 groups depending on distribution of answers from each question, data from food records was split into comparable groups, and Kendall's tau-b rank correlation coefficient or Chi-Square test was used to further examine association between the two methods. Additionally, in paper II, the computer program SAS version 9.1 was used to perform a nonparametric Jonckheere-Terpstra trend test, to see if categories according to the AGES-FFQ ranked mean intake from the food record in an anticipated, graded order. Significance level was set at $p \leq 0.05$.

4.5.2.2 Papers III & IV

Statistical analysis was done using SAS (version 9.2; SAS Institute Inc., Cary, NC, USA). Characteristics of study participants were described using mean and standard deviation of normal variables, median and interquartile range for skewed variables and percentages for dichotomous variables. Univariate and multivariate linear regression was used for examining the association between milk/cod liver oil intake at different periods of life and hip BMD in old age. The lowest intake group (≤ once/week) was in all cases used as referent and results are represented as difference in z-score (Δ) with higher frequency of consumption compared to the referent. Student's t-test was used to test whether BMD was linearly related to consumption (ordinal values). Visual inspection of model residual suggested that use of z-scores was justifiable.

Data are presented unadjusted and adjusted for age, past and present physical activity, alcohol consumption, and midlife or current BMI. Midlife BMI was chosen as a covariate for the retrospective data, and current BMI for current data. Alcohol consumption was divided into <25g/week, 25-50g/week, and >50g/week. Furthermore, in paper III we also adjusted for cod liver oil intake at the same period of life, and in paper IV we adjusted for milk consumption.

For stability analyses individuals taking medication known to affect bone health at the time of AGES examinations, 435 men (21%) and 992 women (37%), were excluded. The medications that resulted in exclusion for this secondary analysis were antiepileptic medication, calcium supplements, oral estrogens, glucocorticoids, osteoporosis drugs, prostate disease drugs, proton pump inhibitors, oral steroids and thyroid agonists.

4.6 Approvals

For the first validation study, participants handed in signed informed written consent along with their completed AGES-FFQs.

Data for the second validation study was gathered in the IceProQualita study, which was approved by the Icelandic National Bioethics Committee (VSNb2008060007/03-15).

The AGES-Reykjavik Study was approved by the Icelandic National Bioethics Committee (VSN: 00-063) and the MedStar IRB for the Intramural Research Program, Baltimore, MD.

5. Results and discussion

5.1. Validity of questions on recalled diet

Detailed dietary data gathered from middle aged individuals in the 1990 INDS was used to assess the validity of questions on recalled diet in midlife (AGES-FFQ), answered by the same individuals 18-19 years later. The part of the AGES-FFQ on midlife diet contains seventeen questions, eleven of which were assessed here. Association between intakes according to the two methods is shown in Table 3. Answers to questions on meat, fish, potatoes, milk and dairy products and cod liver oil were found to have an acceptable correlation to the reference method ($r=0.26-0.56$) for both genders. The strongest correlation was seen for cod liver oil for both genders and milk/dairy for men ($r > 0.4$). Additionally, questions on whole-wheat bread, oatmeal/muesli and blood/liver sausage were found to be reasonably acceptable for men ($r=0.28-0.40$) as well as the question on fresh fruit consumption for women ($r=0.31$; $p=0.001$). The questions on rye bread and vegetable consumption were not valid for either gender.

The frequency and pattern of consumption seem to greatly affect how people remember past diet, and foods consumed daily/several times per day (e.g., milk) may be reported more accurately than those consumed sporadically/several times per month/week (e.g., poultry). Also, consumption of dietary supplements with special characteristics (such as cod liver oil) may be recalled especially well [145]. As reported in the 1990 INDS the diet of Icelanders in that time was characterized by high intake of animal-based products, such as meat, fish, milk and dairy products, a common intake of cod liver oil, and relatively low intake of fruits, vegetables and cereals rich in fibre [15]. The results in the present study were therefore in line with expected results.

Although we did not find the question on vegetable to be valid for either gender, and the question on fruit consumption only had acceptable correlation for women, previous studies on present intake among Icelanders, both children and adults, have yielded better results [129,146]. However, low intake and limited distribution of answers to the AGES-FFQ may have contributed to the low correlation seen between the methods here. Another possible reason for the low correlation may be due to the extreme weight difference between certain types of vegetables, such as leafy vegetables and tubers.

Table 3. Consumption of participants in the 1990 survey compared to reported consumption in midlife according to the AGES-FFQ. Correlation between data in the form of times per week

	1990 g/d			1990 times per week			FFQ times per week				
	P10	median	P90	P10	median	P90	P10	median	P90	correlation	p-value
Men											
Meat	57	138	249	2.6	6.5	12.3	1.5	3.5	5.5	0.30	0.016
Fish	21	89	155	1.1	3.8	7.8	1.5	3.5	3.5	0.26	0.037
Potato	58	146	314	3.4	8.5	18.3	4.3	7.0	7.0	0.35	0.005
Fruit	0	14	145	0	0.8	9.8	0.3	1.5	4.7	0.18	0.168
Blood-/liver sausage	0	0	45	0	0	4.5	0.3	0.3	1.5	0.34	0.007
Rye bread/flatbread	0	0	33	0	0	5.8	0.3	1.5	5.5	0.15	0.224
Whole-wheat bread	27	78	169	3.5	10	22.0	3.5	5.5	7.0	0.40	0.001
Oatmeal/muesli	0	0	97	0	0	6.8	0	0.3	7.0	0.28	0.026
Vegetable	8	33	111	0.6	2.3	7.8	0.3	1.5	5.5	0.15	0.222
Milk and dairy	112	509	1022	1.8	8.8	18.5	1.5	7.0	14.0	0.43	<0.001
Fish liver oil	0	0	13	0	0.3	7.0	0	3.5	7.0	0.53	<0.001
Women											
Meat	41	85	147	1.8	3.7	6.7	1.5	3.5	5.5	0.26	0.009
Fish	28	57	96	1.1	2.3	4.4	1.5	3.5	3.5	0.28	0.004
Potato	29	92	183	1.7	5.4	10.7	3.5	7.0	7.0	0.26	0.008
Fruit	0	69	215	0	5.0	15.1	0.3	3.5	7.0	0.31	0.001
Blood-/liver sausage	0	5	30	0	0.5	3.0	0.3	0.3	1.5	0.21	0.029
Rye bread/flatbread	0	3	35	0	0.5	6.1	0.3	3.5	7.0	0.07	0.507
Whole-wheat bread	19	60	109	2.5	7.8	14.2	3.5	7.0	7.0	0.05	0.582
Oatmeal/muesli	0	0	75	0	0	5.3	0	1.5	7.0	0.22	0.027
Vegetable	18	64	163	1.2	4.5	11.4	0.3	3.5	7.0	0.08	0.418
Milk and dairy	74	336	677	1.1	5.8	12.0	0.3	7.0	7.0	0.29	0.003
Fish liver oil	0	0	13	0	0	7.0	0	3.5	7.0	0.56	<0.001

Table 4. Cross-classification of participants according to midlife intake, proportion in same/adjacent group or grossly misclassified.

	Same category % (N)		**Same or adjacent category % (N)**		**Grossly misclassified % (N)**	
	Men	Women	Men	Women	Men	Women
Meat	20.0 (13)	40.8 (42)	53.9 (35)	85.4 (88)	1.5 (1)	1.0 (1)
Fish	32.3 (21)	48.6 (51)	78.5 (51)	91.4 (96)	0 (0)	1.0 (1)
Potatoes	58.7 (37)	35.6 (37)	81.0 (51)	70.2 (73)	1.6 (1)	1.0 (1)
Fruits	31.7 (20)	22.8 (23)	65.1 (41)	56.4 (57)	6.3 (4)	4.0 (4)
Blood/liver sausage	60.9 (39)	51.0 (53)	53 (82.8)	89.4 (93)	3.1 (2)	1.9 (2)
Rye bread	15.6 (10)	22.3 (23)	62.5 (40)	54.4 (56)	1.6 (1)	7.8 (8)
Whole-wheat bread	47.7 (31)	47.6 (50)	53 (81.5)	72.4 (76)	3.1 (2)	1.9 (2)
Oatmeal/muesli	49.2 (32)	41.9 (44)	66.2 (43)	68.6 (72)	9.2 (6)	10.5 (11)
Vegetables	18.5 (12)	19.8 (21)	52.3 (34)	43.4 (46)	7.7 (5)	8.5 (9)
Milk/dairy products	43.1 (28)	32.0 (33)	72.3 (47)	62.1 (64)	1.5 (1)	4.9 (5)
Cod liver oil	50.0 (31)	46.6 (48)	64.5 (40)	61.2 (63)	12.9 (8)	13.6 (14)

To further assess the relationship between the two methods, cross-classification was performed, comparing individuals categorized into five different groups depending on level of consumption according to the two different methods (Table 4). The percentage of participants classified into the same group by both methods was 16-59% (average 37%), 43-91% (average 69%) into the same or adjacent group and 0-14% (average 4%) were grossly misclassified into the opposite group. Interestingly, cod liver oil, which was most highly correlated to the reference method, was also most likely to be grossly misclassified in the cross-classification. Participants commonly reported daily intake according to the AGES-FFQ while the 1990 INDS revealed no intake. A plausible explanation is that cod liver oil is used seasonally by many Icelanders, as a source of vitamin D during the dark winter months. The diet history gathered in the 1990 INDS was designed to reflect the diet during the past 3 months, while the AGES-FFQ should reflect the usual diet during a whole decade. It is therefore plausible that participants interviewed in the summer of 1990 were not taking cod liver oil at that time, thought it may have been a part of their daily diet most of the year.

5.2 Validity of questions on current diet

Answers to questions on current diet were compared to data from the reference method, the 3-day weighed food record (Table 5). The foods showing the highest correlation were not in all cases the same for men and women, and men generally had higher correlations. For women, correlation ≥ 0.4 was found for rye bread, oatmeal/muesli, raw vegetables, candy, milk and dairy products, pure fruit juice, coffee, tea and cod liver oil (r=0.40-0.61).

Table 5. Correlation between grams of intake from food records and calculated intake from the AGES-FFQ on current diet.

	Food record g/d			AGES-FFQ g/d				
	P10	**median**	**P90**	**P10**	**median**	**P90**	**correlation**	**p-value**
Men (n=53)								
Meat	7	90	248	43	100	100	0.21	0.124
Fish	20	77	190	36	85	114	0.23	0.098
Fish toppings	0	0	23	2	10	23	0.23	0.146
Potatoes	22	74	174	24	86	110	0.46	<0.001
Fresh fruits	0	87	226	24	86	110	0.50	<0.001
Blood/liver sausage	0	0	13	0	3	17	0.05	0.746
Rye bread/flatbread	0	17	59	2	25	50	0.17	0.219
Whole-wheat bread	11	45	73	11	50	50	0.19	0.169
Oatmeal/muesli	0	0	227	0	95	190	0.46	0.001
Cooked vegetables	0	29	113	3	19	60	0.17	0.221
Raw vegetables	0	33	150	3	19	90	0.33	0.015
Cakes and cookies	0	51	137	10	15	70	0.41	0.002
Candy	0	0	13	0	8	18	0.40	0.003
Dairy products	0	84	241	7	103	205	0.55	<0.001
Milk	0	133	560	0	83	264	0.49	<0.001
Pure fruit juice	0	0	192	0	34	160	0.50	<0.001
Soft drink and sweet juice	0	0	134	0	12	231	0.19	0.177
Cod liver oil	0	0	8	0	6	6	0.51	<0.001
Coffee*	87	357	793	105	735	1155	0.63	<0.001
Tea*	0	0	200	0	110	330	0.71	<0.001
Sugar in coffee/tea*	0	0	11	0	0	8	0.53	<0.001
Women (n=75)								
Meat	21	59	151	29	29	68	0.11	0.361
Fish	21	55	137	28	65	102	-0.02	0.873
Fish toppings	0	0	28	0	3	35	0.37	0.001
Potatoes	18	60	116	18	67	85	0.01	0.969
Fresh fruits	39	127	316	60	120	240	0.36	0.001
Blood/liver sausage	0	0	14	0	2	13	0.37	0.001
Rye bread/flatbread	0	7	40	2	30	60	0.42	<0.001
Whole-wheat bread	1	35	76	10	45	45	0.28	0.017
Oatmeal/muesli	0	52	159	3	75	150	0.48	<0.001
Cooked vegetables	0	25	90	3	45	90	0.20	0.089
Raw vegetables	0	53	141	17	40	80	0.40	<0.001
Cakes and cookies	0	36	123	2	30	60	0.20	0.087
Candy	0	0	23	1	6	30	0.43	<0.001
Dairy products	0	100	217	6	85	170	0.50	<0.001
Milk	0	113	369	0	29	135	0.45	<0.001
Pure fruit juice	0	0	167	0	34	160	0.49	<0.001
Soft drink and sweet juice	0	0	148	0	8	48	0.19	0.104
Cod liver oil	0	2	9	0	7	7	0.42	<0.001
Coffee*	67	283	647	98	293	683	0.44	<0.001
Tea*	0	0	363	0	360	840	0.61	<0.001
Sugar in coffee/tea*	0	0	3	0	0	0	0.30	0.008

* portions per day

Furthermore, a correlation between 0.3 and 0.37 was found for fish toppings, fresh fruit, blood/liver sausage, and sugar in coffee/tea. The correlation for whole-wheat bread was lower, but still significant (r=0.28, p=0.017). For men a correlation ≥ 0.4 was found for potatoes, fresh fruits, oatmeal/muesli, cakes/cookies, candy, milk and dairy products, pure fruit juice, cod liver oil, coffee, tea and sugar in coffee/tea (r=0.40-0.71) and a correlation of 0.33 for raw vegetables. The correlation was not significant for fish, meat, cooked vegetables and soft drinks/sweetened juices.

The association between the dietary intake methods was further assessed using a Pearson Chi-Square/Kendall's tau-b (Table 6) and a Jonckheere-Terpstra trend test (Table 7a-b). Results from these tests were largely in agreement with the Spearman's rank test, exceptions being that both tests also showed a significant association for fish topping for men.

Table 6. Association between the two different dietary assessment methods using Chi-Square or Kendall's tau-b.

	Men p-value	**Women p-value**
Meat*	0.389	0.762
Fish*	0.226	0.449
Fish toppings*	0.034	0.008
Potatoes**	<0.001	0.370
Fresh fruits**	<0.001	0.007
Blood/liver sausage*	0.488	0.001
Rye bread/flatbread**	0.084	<0.001
Whole-wheat bread**	0.226	0.005
Oatmeal/muesli**	0.015	<0.001
Cooked vegetables**	0.150	0.226
Raw vegetables**	0.204	0.001
Cakes and cookies**	0.004	0.226
Candy*	0.257	0.003
Dairy products**	<0.001	<0.001
Milk**	0.001	<0.001
Pure fruit juice*	<0.001	0.002
Soft drink and sweet juice*	0.105	0.170
Cod liver oil*	<0.001	<0.001
Coffee¥*	<0.001	0.009
Tea¥*	<0.001	<0.001
Sugar in coffee/tea¥*	0.001	0.015

* Pearson Chi-Square
** Kendall's tau-b
¥ Daily consumption

Additionally the Jonckheere-Terpstra test showed a significant trend for cooked vegetables for women, and no association was detected between the methods for consumption of raw vegetables and candy for men when using Chi Square/Kendall's tau-b. Limited and/or skewed distribution of answers from the AGES-FFQ may contribute to these differences.

Table 7a. Average consumption in g/d (SD) for men depending on answers given in the AGES-FFQ on current diet.

	Frequency of consumption					
Men	**1***	**2†**	**3‡**	**4§**	**5¶**	**trend****
Meat	73 (59)	93 (62)	108 (91)	207 (74)		0.300
	n = 2	n = 19	n = 28	n = 4	n = 0	
Fish	0 (.)	76 (41)	93 (69)	156 (94)		0.093
	n = 1	n = 15	n = 32	n = 5	n = 0	
Fish topping/salad	1 (4)	7 (12)	14 (23)	0 (.)	5 (8)	0.025
	n = 21	n = 19	n = 8	n = 1	n =3	
Potatoes		54 (63)	52 (22)	87 (56)	113 (51)	<0.001
	n = 0	n = 6	n = 6	n = 16	n = 25	
Fresh fruit	0 (.)	40 (63)	60 (49)	165 (93)	153 (95)	<0.001
	n = 2	n = 8	n = 15	n = 9	n = 18	
Blood/liver sausage	3 (13)	7 (22)	0 (.)	0 (.)	14 (.)	0.534
	n = 38	n = 12	n = 1	n =1	n = 1	
Rye bread	7 (12)	22 (23)	23 (21)	36 (28)	26 (39)	0.108
	n = 9	n = 14	n = 17	n = 4	n = 8	
Whole wheat bread	15 (21)	40 (28)	48 (42)	41 (21)	50 (28)	0.036
	n = 2	n = 6	n = 9	n = 8	n = 27	
Oatmeal/muesli	6 (24)	51 (61)	40 (52)	83 (63)	115 (128)	0.001
	n = 14	n = 8	n = 10	n = 4	n = 17	
Cooked vegetables	33 (58)	38 (45)	57 (63)	33 (28)	92 (.)	0.112
	n = 10	n = 26	n = 11	n = 4	n = 1	
Fresh vegetables	10 (15)	57 (57)	47 (56)	66 (29)	102 (78)	0.011
	n = 7	n = 20	n = 15	n = 5	n = 6	
Cakes and cookies	38 (40)	42 (39)	55 (54)	116 (95)	112 (86)	0.012
	n = 12	n = 14	n = 10	n = 6	n = 10	
Candy	0 (1)	9 (15)	12 (31)	3 (4)		0.009
	n = 24	n = 15	n = 10	n = 4	n = 0	
Dairy products	20 (49)	64 (62)	88 (54)	80 (44)	249 (145)	<0.001
	n = 7	n = 14	n = 15	n = 7	n = 10	
Milk	90 (103)	184 (105)	209 (232)	132 (115)	285 (222)	0.001
	n = 20	n = 5	n = 7	n = 2	n = 19	
Pure fruit juice	8 (30)	20 (60)	45 (86)	129 (155)	126 (114)	<0.001
	n = 24	n = 9	n = 7	n = 3	n = 9	
Soft drink/sweet juice	65 (218)	49 (61)	113 (58)	60 (70)	0 (.)	0.073
	n = 34	n = 9	n = 4	n = 4	n = 1	
Cod liver oil	0 (0)	0 (.)	6 (.)	0.1 (0.2)	3 (3)	<0.001
	n = 14	n = 1	n = 1	n = 3	n = 34	
Coffee††	122 (95)	298 (169)	450 (211)	529 (310)	792 (227)	<0.001
	n = 8	n = 14	n = 23	n = 5	n = 3	
Tea††	30 (58)	154 (114)	206 (123)			<0.001
	n = 39	n = 11	n = 3	n=0	n=0	
Sugar††	1 (4)	18 (21)	0 (.)			<0.001
	n = 46	n = 6	n = 1	n=0	n=0	

Table 7b. Average consumption in g/d (SD) for women depending on answers given in the AGES-FFQ.

	Frequency of consumption					
Women	**1***	**2†**	**3‡**	**4§**	**5¶**	**trend****
Meat	52 (68)	73 (48)	70 (41)	92 (37)	0	0.236
	n = 6	n = 36	n = 30	n = 3	n = 0	
Fish	16 (23)	71 (48)	68 (40)	57 (49)		0.874
	n = 2	n = 28	n = 37	n = 8	n = 0	
Fish topping/salad	3 (10)	7 (16)	12 (13)	45 (7)	36 (1)	<0.001
	n = 45	n = 22	n = 4	n = 2	n = 2	
Potatoes	72 (61)	74 (34)	54 (39)	73 (34)	58 (32)	0.631
	n = 3	n = 8	n = 23	n = 18	n = 23	
Fresh fruit	0 (0)	78 (45)	100 (47)	111 (46)	175 (122)	0.002
	n = 1	n = 5	n = 6	n = 13	n = 50	
Blood/liver sausage	2 (10)	7 (16)	16 (17)			0.001
	n = 57	n = 15	n = 3	n = 0	n = 0	
Rye bread	7 (16)	4 (7)	28 (28)	18 (21)	23 (16)	<0.001
	n = 16	n = 18	n = 20	n = 8	n = 13	
Whole wheat bread	26 (46)	51 (46)	32 (20)	34 (37)	47 (29)	0.012
	n = 5	n = 8	n = 14	n = 9	n = 39	
Oatmeal/muesli	12 (26)	27 (34)	62 (50)	198 (137)	92 (67)	<0.001
	n = 14	n = 12	n = 16	n = 3	n = 28	
Cooked vegetables	44 (43)	24 (32)	28 (34)	41 (28)	73 (56)	0.049
	n = 10	n = 25	n = 24	n = 6	n = 10	
Fresh vegetables	20 (21)	50 (39)	46 (41)	90 (55)	93 (64)	0.003
	n = 5	n = 19	n = 21	n = 15	n = 15	
Cake and cookie	36 (45)	49 (31)	39 (55)	42 (33)	71 (49)	0.1214
	n = 13	n = 23	n = 17	n = 6	n = 16	
Candy	1 (2)	9 (21)	11 (15)	7 (5)	13 (11)	<0.001
	n = 24	n = 24	n = 15	n = 4	n = 8	
Dairy products	22 (37)	88 (76)	123 (92)	114 (74)	159 (83)	<0.001
	n = 10	n = 19	n = 13	n = 15	n = 18	
Milk	95 (132)	107 (80)	126 (25)	8 (11)	225 (123)	<0.001
	n = 29	n = 11	n = 4	n = 2	n = 29	
Pure fruit juice	3 (8)	39 (71)	26 (47)	73 (86)	101 (93)	<0.001
	n = 25	n = 15	n = 10	n = 9	n = 16	
Soft drink/sweet juice	32 (71)	45 (75)	108 (74)			0.081
	n = 61	n = 10	n = 4	n = 0	n = 0	
Cod liver oil	0.2 (0.5)		0 (.)	11 (13)	5 (5)	0.004
	n = 11	n = 0	n = 1	n = 4	n = 56	
Coffee††	125 (105)	307 (164)	376 (214)	522 (350)		<0.001
	n = 11	n = 30	n = 30	n = 4	n = 0	
Tea††	23 (51)	189 (169)	249 (294)	133 (.)		<0.001
	n = 36	n = 30	n = 8	n = 1	n = 0	
Sugar††	1 (2)	5 (9)				0.025
	n = 71	n = 3	n = 0	n = 0	n = 0	

* 1: never or less than once a week
† 2: 1-2 times a week
‡ 3: 3-4 times a week
§ 4: 5-6 times a week
¶ 5: daily or more than once a day
** Jonckheere-Terpstra trend test
†† Consumption in times per day instead of per week

Part of the explanation for low or no correlation in general seen between the two dietary intake methods may be the inability of a 3-day food record to adequately reflect individuals' intake of foods that are consumed infrequently, such as the soft drinks/sweetened juices in the present study. As the food in question may not show up on the 3-day food record the AGES-FFQ might reflect more accurate intake, and may be better suited to rank individuals according to level of consumption. A longer period of food recording, or repeated recordings, might have increased the likelihood of finding a correlation to such questions.

Meat, fish and cooked vegetables were however not consumed infrequently (average of 2-4 times per week), but a possible explanation for the insignificant correlation to the reference method may lie in the lack of distribution of answers to the AGES-FFQ. Regarding the questions on meat and fish consumption, almost 90% of our participants marked either of two options – 1-2 times a week or 3-4 times a week – as did 70% when it came to the question on cooked vegetables. Global questions, chosen for the sake of simplicity, may thus limit the validity of the AGES-FFQ. The questions with narrow distribution of answers, such as for meat, fish and vegetables, could presumably be improved by increasing frequency options to improve distribution, as well as by splitting them up into separate questions e.g., on types of meat, fish, etc.

The reference method used for assessing the validity of questions on midlife diet may have been better able to detect correlation for food consumed <2-3 times a week than the 3-day weighed food recording used here. In the previous study there was a significant correlation between methods for fish and meat consumption (r=0.25-0.30), along with a stronger correlation for cod liver oil. Part of the explanation for higher correlation in some cases might be due to the fact that the reference method, the detailed dietary history, reflects long-term diet. Another possible explanation might be that both dietary assessment methods can be subject to similar sources of error, such as bias to overestimate foods considered healthy, and to underestimate foods considered unhealthy. No clear sign of over-/underestimation was found in the present study, neither related to gender nor foods/food groups considered healthy/unhealthy. However, foods consumed infrequently according to the AGES-FFQ may not have shown up in the 3-day food records and lead to the perception of overestimation according to the AGES-FFQ.

5.3 Association between lifelong milk consumption and bone mineral density in old age

Possible confounders are shown in Table 8 in relation to category of milk consumption in adolescence, midlife and current old age. Average frequency of milk consumption was high at all periods of life, although the proportion of participants reporting any intake decreased with age. Only 1.5% reported no intake in adolescence, 3.5% in midlife and 14% reported no current consumption. Daily consumption decreased from 77% in adolescence to 59% in midlife and 49% in current old age (data not shown).

Integral BMD (g/cc) of the femoral neck and trochanter, is shown in Table 9.

Table 8. Possible confounding factors in relation to milk consumption in adolescence (14-19y), midlife (40-50y) and current consumption. Data shown as mean (SD), median (IQR), or proportion (%).

		Men			Women		
		<once/week	**1-6/week**	**≥once/day**	**<once/week**	**1-6/week**	**≥once/day**
Adolescence	Age, y	77.5 (6.2)	76.5 (5.2)	76.4 (5.4)	76.4 (6.2)	75.7 (5.3)	76.2 (5.6)
	Midlife BMI, kg/m^2	26.1 (3.0)	25.6 (3.0)	25.5 (3.2)	24.8 (6.1)*	24.4 (4.7)*	24.2 (4.4)*
	Current BMI, kg/m^2	26.8 (3.9)	26.8 (3.7)	26.8 (3.8)	28.2 (6.9)*	27.1 (6.0)*	26.7 (5.9)*
	Alcohol, g/week	3.2 (30.8)*	6.4 (26.4)*	6.4 (26.4)*	1.6 (6.4)*	1.6 (9.7)*	1.6 (8.0)*
	PA present, hours/week	0.03 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.0 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
	PA 50-65y, hours/week	0.0 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
	PA 35-49y, hours/week	0.0 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
	PA 20-34y, hours/week	0.05 (0.5)*	0.05 (1.5)*	0.05 (5.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
	Daily intake of cod liver oil	30%	21.7%	32.9%	20%	23.8%	38.8%
	Medications**	22.5%	21.8%	20.4%	43.2%	36.7%	36.4%
Midlife	Age, y	76.6 (5.6)	76.1 (5.2)	76.6 (5.4)	76.5 (5.8)	75.6 (5.2)	76.4 (5.6)
	Midlife BMI, kg/m^2	25.8 (3.2)	25.6 (3.2)	25.4 (3.1)	24.5 (4.9)*	24.3 (4.4)*	24.2 (4.5)*
	Current BMI, kg/m^2	27.2 (4.1)	27.0 (3.7)	26.6 (3.8)	27.5 (6.3)*	27.0 (5.9)*	26.6 (5.9)*
	Alcohol, g/week	6.4 (33.0)*	8.0 (24.8)*	4.8 (26.4)*	1.6 (16.1)*	1.6 (9.7)*	1.6 (8.0)*
	PA present, hours/week	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.0 (0.05)*	0.05 (1.5)*	0.0 (1.5)*
	PA 50-65y, hours/week	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
	PA 35-49y, hours/week	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
	PA 20-34y, hours/week	0.05 (1.5)*	0.05 (5.5)*	0.05 (5.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
	Daily intake of cod liver oil	42.1%	34.4%	48.9%	37.8%	39.6%	46.2%
	Medications**	23.6%	19.6%	20.9%	35.3%	36.9%	37.0%
Current	Age, y	76.3 (5.4)	75.9 (5.1)	76.8 (5.4)	76.0 (5.6)	75.9 (5.3)	76.3 (5.6)
	Midlife BMI, kg/m^2	25.4 (3.4)	25.6 (3.0)	25.5 (3.1)	24.2 (4.4)*	24.3 (4.4)*	24.4 (4.5)*
	Current BMI, kg/m^2	26.5 (3.9)	27.0 (3.6)	26.8 (3.8)	26.6 (6.0)*	26.8 (6.1)*	26.9 (5.8)*
	Alcohol, g/week	8.0 (32.8)*	8.0 (24.8)*	4.8 (24.1)*	3.2 (13.2)*	1.6 (8.0)*	1.6 (6.4)*
	PA present, hours/week	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.0 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
	PA 50-65y, hours/week	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
	PA 35-49y, hours/week	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
	PA 20-34y, hours/week	0.05 (5.5)*	0.05 (4.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
	Daily intake of cod liver oil	59.8%	55.6%	63.6%	54.2%	57.5%	65.7%
	Medications**	19.8%	17.0%	22.7%	35.9%	36.1%	37.7%

*median and interquartile range; **Medications: Current intake of medication known to affect bone density

Table 9. QCT measurements of left femoral neck and trochanteric area.

	Men			Women		
	P10	**P50**	**P90**	**P10**	**P50**	**P90**
Femoral neck integral BMD (g/cc)	0.19	0.25	0.32	0.18	0.24	0.31
Trochanter integral BMD (g/cc)	0.19	0.25	0.32	0.17	0.22	0.29

5.3.1 Association between milk consumption and BMD

Both midlife and current milk consumption was highly correlated to the reference methods according to the validation studies. BMD of individuals with medium and high frequency of milk consumption was compared to those with low frequency of consumption and difference in BMD shown as difference in Z-scores.

The association between milk intake and hip BMD is shown in Tables 10 and 11. Data is shown separately for men and women and both unadjusted and adjusted for confounders. Individuals with the highest frequency of consumption in adolescence (≥once/day) had Z-scores approximately 0.08-0.15 higher in old age, compared to those with the lowest frequency of consumption (<once/week), the difference was insignificant for both femoral neck and trochanter, and in both genders. Men with the highest frequency of milk consumption during midlife had significantly higher Z-scores by 0.21 and 0.22 for femoral neck and trochanter respectively, compared to those with the lowest frequency of consumption. Comparable differences in Z-scores for women were 0.20 and 0.18 for femoral neck and trochanter respectively. Those women with midlife consumption of 1-6 times/week also had significantly higher Z-scores for femoral neck compared to women in the lowest intake group. For current consumption, individuals with the highest frequency of consumption had Z-scores approximately 0.09 higher than those with the lowest frequency of consumption. While the difference was only significant for trochanter for women, the trend was significant for both femoral neck and trochanter for men.

The stronger association seen for men compared to women was somewhat surprising, as bone loss is generally thought to be more gradual for men than women [97,98]. It is possible that the stronger association might be related to the validity of the AGES-FFQ, as men's answers to questions on milk consumption were of greater validity than those for women. The association seen for men may therefore reflect an even more accurate relationship than for women. It is likely that calcium supplementation and/or increased milk consumption among men has comparable effect as among women, but intervention studies have shown that calcium supplementation in elderly women can have a small benefit on age-related bone loss [100,101], and milk supplementation may diminish bone turnover among postmenopausal women [102].

Table 10. Difference in Z-scores, derived from femoral neck BMD, between individuals consuming milk consumption of 1-6 times/week and ≥once/day compared to <once/week.

	Femoral neck			
	Men		Women	
	Δ Z	**95% CI**	**Δ Z**	**95% CI**
Unadjusted				
Adolescence				
<once/week	referent	-	-	-
1-6 times/week	0.11	-0.21 ; 0.44	0.10	-0.11 ; 0.30
≥once/day	0.15	-0.16 ; 0.46	0.08	-0.12 ; 0.27
p for trend		0.29		0.85
Midlife				
<once/week	referent	-	-	-
1-6 times/week	0.17	-0.02 ; 0.35	0.17	0.02 ; 0.31
≥once/day	0.20	0.02 ; 0.37	0.17	0.03 ; 0.30
p for trend		0.05		0.09
Current				
<once/week	referent	-	-	-
1-6 times/week	-0.01	-0.13 ; 0.12	0.06	-0.05 ; 0.16
≥once/day	0.08	-0.02 ; 0.19	0.06	-0.03 ; 0.15
p for trend		0.08		0.23
Adjusted*				
Adolescence				
<once/week	referent	-	-	-
1-6 times/week	0.11	-0.21 ; 0.43	0.11	-0.08 ; 0.30
≥once/day	0.15	-0.16 ; 0.45	0.12	-0.06 ; 0.30
p for trend		0.29		0.31
Midlife				
<once/week	referent	-	-	-
1-6 times/week	0.15	-0.02 ; 0.33	0.14	0.002 ; 0.27
≥once/day	0.21	0.04 ; 0.38	0.20	0.07 ; 0.33
p for trend		0.02		0.002
Current				
<once/week	referent	-	-	-
1-6 times/week	-0.02	-0.14 ; 0.10	0.06	-0.03 ; 0.16
≥once/day	0.09	-0.01 ; 0.20	0.07	-0.01 ; 0.16
p for trend		0.04		0.12

*Data adjusted for age, past and present physical activity, alcohol consumption, cod liver oil intake at the same period, midlife BMI for adolescence and midlife consumption and current BMI for current consumption

Table 11. Difference in Z-scores, derived from BMD of trochanteric area, between individuals consuming milk 1-6 times/week and ≥once/day compared to <once/week.

	Trochanteric area			
	Men		Women	
	Δ Z	95% CI	Δ Z	95% CI
Unadjusted				
Adolescence				
<once/week	-	-	-	-
1-6 times/week	0.02	-0.30 ; 0.34	0.10	-0.11 ; 0.30
≥once/day	0.09	-0.22 ; 0.40	0.09	-0.10 ; 0.28
p for trend		0.19		0.68
Midlife				
<once/week	-	-	-	-
1-6 times/week	0.16	-0.03 ; 0.34	0.17	0.03 ; 0.31
≥once/day	0.20	0.02 ; 0.37	0.15	0.01 ; 0.28
p for trend		0.04		0.23
Current				
<once/week	-	-	-	-
1-6 times/week	0.05	-0.07 ; 0.17	0.09	-0.02 ; 0.19
≥once/day	0.09	-0.02 ; 0.19	0.08	-0.01 ; 0.17
p for trend		0.11		0.11
Adjusted*				
Adolescence				
<once/week	-	-	-	-
1-6 times/week	0.004	-0.31 ; 0.32	0.11	-0.08 ; 0.29
≥once/day	0.08	-0.22 ; 0.38	0.13	-0.04 ; 0.31
p for trend		0.17		0.17
Midlife				
<once/week	-	-	-	-
1-6 times/week	0.15	-0.03 ; 0.32	0.13	-0.004 ; 0.26
≥once/day	0.22	0.05 ; 0.38	0.18	0.05 ; 0.30
p for trend		0.008		0.007
Current				
<once/week	-	-	-	-
1-6 times/week	0.03	-0.09 ; 0.15	0.09	-0.002 ; 0.18
≥once/day	0.09	-0.01 ; 0.20	0.09	0.01 ; 0.17
p for trend		0.05		0.04

*Data adjusted for age, past and present physical activity, alcohol consumption, cod liver oil intake at the same period, midlife BMI for adolescence and midlife consumption and current BMI for current consumption

5.3.2 Clinical relevance

It has been estimated that a 1 SD decrease in hip BMD is associated with approximately 2.5 fold increased risk of hip fracture [147,148]. Thus the difference of 0.2 Z-scores (equal to 0.2 SD) seen between individuals with high versus low frequency of milk intake in midlife may be considered of great clinical significance, possibly associated with a 20-30% increased risk for those in the lowest intake group, assuming linear relationship between BMD and fracture risk.

Table 12. Possible confounding factors in relation to cod liver oil intake in adolescence (14-19y), midlife (40-50y) and current old age. Data shown as mean (SD), median (IQR), or proportion (%).

		Men			Women		
		<once/week	**1-6 times/week**	**Daily**	**<once/week**	**1-6 times/week**	**Daily**
Adolescence	Age, y	76.6 (5.5)	76.0 (5.3)	76.6 (5.1)	76.1 (5.7)	75.6 (5.3)	76.4 (5.4)
	Midlife BMI, kg/m^2	25.4 (3.0)	25.7 (3.3)	25.5 (3.1)	24.4 (4.6)*	23.7 (4.2)*	24.4 (4.4)*
	Current BMI, kg/m^2	26.7 (3.8)	27.0 (4.0)	26.7 (3.7)	26.8 (6.2)*	26.4 (5.7)*	27.0 (5.7)*
	Alcohol, g/week	6.4 (26.4)*	8.0 (26.4)*	4.8 (24.1)*	1.6 (8.0)*	1.6 (8.0)*	1.6 (8.0)*
	PA current, hours/week	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.0 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
	PA 50-65y, hours/week	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
	PA 35-49y, hours/week	0.05 (1.5)*	0.05 (1.5)*	0.05 (5.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
	PA 20-34y, hours/week	0.05 (1.5)*	0.05 (5.5)*	0.5 (5.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
	Daily milk intake	78.4%	73.2%	84.7%	71.0%	72.1%	83.9%
	Medications**	21.4%	19.5%	20.6%	37.1%	35.6%	37.0%
Midlife	Age, y	76.9 (5.6)	75.4 (5.3)	76.6 (5.1)	76.1 (5.6)	75.6 (5.3)	76.3 (5.5)
	Midlife BMI, kg/m^2	25.7 (3.3)	25.4 (3.1)	25.4 (3.0)	24.6 (4.6)*	23.8 (4.3)*	24.2 (4.4)*
	Current BMI, kg/m^2	27.1 (4.0)	27.0 (3.8)	26.4 (3.6)	27.1 (6.0)*	26.4 (6.1)*	26.6 (5.8)*
	Alcohol, g/week	4.8 (26.4)*	8.0 (26.4)*	6.4 (26.4)*	1.6 (8.0)*	3.2 (8.0)*	1.6 (8.0)*
	PA current, hours/week	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.0 (0.5)*	0.05 (1.5)*	0.05 (1.5)*
	PA 50-65y, hours/week	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
	PA 35-49y, hours/week	0.05 (1.5)*	0.05 (1.5)*	0.05 (5.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
	PA 20-34y, hours/week	0.05 (1.5)*	0.05 (1.5)*	0.5 (5.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
	Daily milk intake	59.0%	52.7%	69.3%	53.7%	52.0%	60.3%
	Medications**	20.7%	19.8%	21.1%	36.3%	38.3%	36.5%
Current	Age, y	76.6 (5.6)	75.4 (5.1)	76.6 (5.3)	76.1 (5.6)	75.4 (5.2)	76.2 (5.5)
	Midlife BMI, kg/m^2	25.6 (3.5)	25.3 (3.0)	25.5 (3.0)	24.8 (4.9)*	24.2 (4.3)*	24.1 (4.3)*
	Current BMI, kg/m^2	27.2 (4.1)	26.9 (3.5)	26.6 (3.7)	27.3 (6.3)*	26.6 (6.6)*	26.5 (5.7)*
	Alcohol, g/week	4.8 (26.4)*	13.2 (24.8)*	6.4 (26.4)*	1.6 (6.4)*	3.2 (9.7)*	1.6 (8.0)*
	PA current, hours/week	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.0 (0.5)	0.05 (1.5)*	0.05 (1.5)*
	PA 50-65y, hours/week	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
	PA 35-49y, hours/week	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
	PA 20-34y, hours/week	0.05 (1.5)*	0.05 (1.5)*	0.05 (5.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
	Daily milk intake	49.1%	47.7%	54.9%	41.6%	37.6%	51.0%
	Medications**	18.1%	20.7%	22.1%	39.1%	34.4%	35.9%

*median and interquartile range; **Medications: Current intake of medication known to affect bone density

5.4 Cod liver oil intake, bone mineral density, and serum 25(OH)D in old age.

Possible confounding factors in relation to cod liver oil intake at different periods of life can be seen in Table 12. Intake of cod liver oil was fairly common and there was a clear trend in cod liver oil consumption with age, proportion of participants reporting any intake being 61%-70%-74% in adolescence, midlife, and current old age respectively, and 33%-43%-60% reported daily intake. This trend is similar to that seen across age groups in INDSs, e.g., in the 2010-1011 INDS 14% of the 18-30year-old, 22% of the 31-60year-old and 34% of the 61-80year-old took cod liver oil daily [17]. The most likely explanation for the lower proportion of elderly individuals with daily intake of cod liver oil in the 2010-2011 INDS compared to the present study is that the AGES-FFQ asks of consumption of both liquid cod liver oil and cod liver oil capsules, while numbers from the INDS are on liquid cod liver oil consumers only.

5.4.1 Retrospective intake of cod liver oil and association with hip bone mineral density in old age

The association between retrospective intake of cod liver oil and difference in Z-scores, calculated from BMD of femoral neck and trochanteric area in old age, are shown in Table 13. Data is shown separately for men and women and both unadjusted and adjusted for confounders. We did not find the intake of cod liver oil during adolescence or midlife to be associated with BMD of either femoral neck or trochanter. It should be noted that we did not have any information on supplement use during midlife, and could therefore not analyse the data separately without supplement users. Hence, we do not know if such an analysis might have yielded different results.

Cod liver oil is not only a generous source of vitamin D, but also of vitamin A in the form of retinol. In the early and mid 20^{th} century cod liver oil was not a standardized product and retinol levels were commonly high. Even up until 2002 cod liver oil in Iceland contained approximately 30.000µg of retinol /100g, while today's levels are 5000µg and 200µg/100g of retinol and vitamin D respectively. High intakes of retinol have repeatedly been linked to adverse effects on bone health and even increased risk of osteoporosis and osteoporotic fractures [78–82].

Cod liver oil is a traditional source of vitamin D in other Nordic countries as well as Iceland, and concentrations have generally been similar [25]. A Norwegian follow-up study of elderly women showed cod liver oil intake in childhood to be associated with significantly lower current forearm BMD, compared to those with no childhood intake [25]. The researchers concluded that the previously high concentration of retinol in cod liver oil, when added to an already vitamin A rich diet, may have lead to total intake reaching harmful levels. We however did not find any indication that the intake of cod liver oil during adolescence or midlife was associated with adverse effects on hip BMD in old age. It should be noted that these two studies use different methods of measuring BMD, as well as measuring different bones, and it is possible that forearm and hip bones respond differently.

Table 13. Difference in Z-scores, derived from hip BMD, between individual with cod liver oil intake of 1-6 times/week or daily intake in adolescence and midlife compared to <once/week.

	Femoral neck				Trochanteric area			
	Men		Women		Men		Women	
	Δ Z	95% CI	Δ Z	95% CI	Δ Z	95% CI	Δ Z	95% CI
Unadjusted								
Adolescence intake								
<once/week	referent	-	-	-	-	-	-	-
1-6 times/week	0.09	-0.01 ; 0.20	-0.09	-0.20 ; 0.01	0.08	-0.03 ; 0.19	-0.10	-0.20 ; 0.01
≥once/day	0.02	-0.08 ; 0.12	0.04	-0.05 ; 0.12	0.01	-0.09 ; 0.11	0.05	-0.03 ; 0.14
p-value		0.61		0.48		0.79		0.28
Midlife intake								
<once/week	referent	-	-	-	-	-	-	-
1-6 times/week	0.02	-0.10 ; 0.14	-0.06	-0.17 ; 0.05	-0.01	-0.13 ; 0.11	-0.06	-0.17 ; 0.06
≥once/day	0.01	-0.09 ; 0.11	0.01	-0.07 ; 0.10	-0.01	-0.11 ; 0.09	0.01	-0.07 ; 0.09
p-value		0.83		0.73		0.84		0.83
Adjusted*								
Adolescence intake								
<once/week	referent	-	-	-	-	-	-	-
1-6 times/week	0.06	-0.05 ; 0.16	-0.10	-0.20 ; 0.01	0.05	-0.05 ; 0.16	-0.09	-0.19 ; 0.01
≥once/day	0.01	-0.09 ; 0.11	0.05	-0.03 ; 0.12	-0.004	-0.10 ; 0.09	0.06	-0.01 ; 0.14
p-value		0.79		0.30		0.94		0.13
Midlife intake								
<once/week	referent	-	-	-	-	-	-	-
1-6 times/week	-0.03	-0.15 ; 0.08	-0.08	-0.19 ; 0.02	-0.06	-0.18 ; 0.06	-0.07	-0.17 ; 0.03
≥once/day	-0.01	-0.11 ; 0.09	0.02	-0.06 ; 0.10	-0.03	-0.13 ; 0.06	0.02	-0.05 ; 0.10
p-value		0.84		0.58		0.53		0.51

*Adjusted for age, past and present physical activity, midlife BMI, alcohol consumption, and milk consumption at the same period

5.4.2 Cod liver oil intake in current old age and association with hip bone mineral density and serum 25(OH)D concentrations

The association between cod liver oil intake in current old age and Z-scores, calculated from BMD of femoral neck and trochanteric area in old age, is shown in Table 14. Intervention studies have shown that vitamin D supplementation can be associated with increased BMD of elderly individuals [103,104]. The few studies existing on cod liver oil intake in old age and BMD have not found an association however [149,150]. In the present study, there was no significant difference in hip BMD in relation to frequency of current cod liver oil intake for men, while women with daily intakes had Z-scores approximately 0.1 higher than those with intake of $\leq$ once/week. Supplement users were excluded from the analysis. The weak association found between current cod liver oil intake and hip BMD in our study may possibly be explained by the relatively high serum 25(OH)D levels in our population, possibly masking any putative benefit of cod liver oil intake.

Current cod liver oil intake was related to serum 25(OH)D concentration (Table 15), those with medium or high intake of cod liver oil ($\geq$ once a week) having significantly higher levels compared to those with the lowest intake (never or less than once a week). Median 25(OH)D concentration in the lowest intake group was 40.2nmol/L and 37.8nmol/L for men and women respectively, and 61.9nmol/L and 56.4nmol/L for men and women in the highest intake group. When excluding supplement users, the main difference was seen in the median serum 25(OH)D levels in individuals with the lowest cod liver oil intake, concentrations being 37.2nmol/L and 31.9nmol/L for men and women respectively. The association between current frequency of intake and serum 25(OH)D concentrations may be considered as further validation of the question on cod liver oil in the AGES-FFQ, as concentrations increased with increased frequency of intake of cod liver oil.

5.4.3 Clinical relevance

As mentioned previously (5.3.2), 1 SD decrease in hip BMD is estimated to be associated with approximately 2.5 fold increased risk of hip fracture [147,148]. Thus the difference of 0.1 Z-scores (equal to 0.1 SD), seen between women with daily intake of cod liver oil versus <once/week, may possibly be of clinical relevance.

Table 14. Difference in Z-scores, derived from hip BMD, in relation to current cod liver oil intake (supplement users excluded).

	Femoral neck				Trochanteric area			
	Men		Women		Men		Women	
	Δ Z	95% CI	Δ Z	95% CI	Δ Z	95% CI	Δ Z	95% CI
Unadjusted								
<once/week	referent	-	-	-	-	-	-	-
1-6 times/week	0.06	-0.11 ; 0.23	-0.02	-0.20 ; 0.15	0.11	-0.06 ; 0.28	-0.06	-0.23 ; 0.12
daily	-0.0003	-0.11 ; 0.11	0.05	-0.05 ; 0.15	0.02	-0.09 ; 0.13	0.03	-0.08 ; 0.13
p-value		0.93		0.34		0.86		0.56
Adjusted*								
<once/week	referent	-	-	-	-	-	-	-
1-6 times/week	0.04	-0.13 ; 0.20	-0.07	-0.23 ; 0.10	0.10	-0.07 ; 0.26	-0.12	-0.28 ; 0.04
daily	-0.02	-0.13 ; 0.09	0.11	0.01 ; 0.20	0.02	-0.09 ; 0.12	0.10	0.01 ; 0.19
p-value		0.69		0.02		0.88		0.02

*Adjusted for age, past and present physical activity, current BMI, alcohol consumption, and current milk consumption

Table 15. Serum 25(OH)D concentration in nmol/L in relation to current cod liver oil intake.

	men				women			
	n	median	P10	P90	n	median	P10	P90
All subjects								
<once/week	642	40.2	20.3	70.8	933	37.8	17.1	67.8
1-6 times/week	256	48.3*	27.8	86.1	249	44.9*	21.6	76.3
daily	1375	61.9*	35.2	93.1	1856	56.4*	29.2	84.4
p-value		<0.001				<0.001		
Without supplement								
<once/week	518	37.2	19.6	67.7	669	31.9	15.2	63.5
1-6 times/week	203	48.9*	28.1	86.6	174	44.0*	21.3	70.1
daily	979	60.6*	33.9	94.5	1168	55.2*	27.7	85.6
p-value		<0.001				<0.001		

* Significantly different from the lowest intake group, p<0.0001

6. Limitations

When assessing the validity of questions on current intake, the inability of a 3-day food record to adequately reflect individual intake of foods that are consumed infrequently, may partly explain cases of low or no correlation between the two methods. For such foods the 3-day food record may not be the ideal reference method and the AGES-FFQ data might even be closer to true intake in these cases. Furthermore, global questions such as those on fish and meat consumption, chosen for the sake of simplicity, may have limited the validity of the AGES-FFQ. Validity of those questions could presumably be improved by splitting them up into separate questions on types of meat, fish, etc. as well as increasing the number of frequency choices in order to increase the distribution of answers.

Although the results from the validation studies show that the AGES-FFQ is able to rank individuals according to their intake of several important food groups, one should always be aware of the limitations of the method and the different results seen for different food items. It should also be noted that the AGES-FFQ is only appropriate for ranking individuals according to level of intake of selected foods and food groups, and not for assessing total intake, energy or specific nutrients.

In relation to the assessment of milk and cod liver oil consumption and hip BMD in old age, the main limitation is that we are partly using retrospective data with 60 years of temporal separation on average, which is always going to be imprecise and is likely to mask any potential modest or weak association. However, studies have shown that there is not necessarily a clear decline in accuracy of reports with increased time lag, and recalled diet from childhood, with 50 years of temporal separation, may be fairly accurate [145]. Furthermore, foods eaten daily, as well as foods, meals or supplements with special characteristics (such as cod liver oil), can be recalled particularly well [145].

When it comes to assessing the association between milk and cod liver oil intake and hip BMD in old age, the inability to accurately assess amounts consumed is also a limitation. Still, the comparisons being made between the lowest intake group (<once/week) and the highest (daily or ≥once/day) can be of practical relevance and ranking individuals according to intake is a common practice in epidemiologic research.

Also, when assessing association between current intake and hip BMD, as well as associations between cod liver oil intake and serum 25(OH)D, we are using cross-sectional data, and it can be expected that several factors, not accounted for in the analysis, affect the association.

7. Conclusion and future studies

The AGES-FFQ can be used to rank individuals according to their intake of several important food groups, including milk and cod liver oil, making it possible to assess association with important health-related outcomes.

Our findings regarding milk consumption at different periods of life and hip BMD in old age, suggest that regular consumption throughout life, from adolescence to old age, is associated with higher BMD in old age. The strongest association was seen for midlife milk consumption, and was stronger for men than women.

The relationship between cod liver oil intake and BMD is less clear. Retrospective intake, in adolescence or midlife, does not appear to be associated with hip BMD in old age; neither does current intake for men. Current intake was however positively associated with BMD in old age for women. The weak association observed between current cod liver oil intake and hip BMD may possibly be explained by the relatively high average serum 25(OH)D levels in our population. Any putative benefit of cod liver oil intake may therefore possibly be masked by these relatively high serum concentrations.

The interest in milk and cod liver oil consumption and its association to BMD is primarily due to the relationship between BMD and osteoporosis and fracture risk. The available data, already gathered as a part of the AGES-Reykjavik study, provides an opportunity to further study possible relationships between intake and health-related outcomes. The association between cod liver oil, serum 25(OH)D concentration and health-related variables, such as muscle strength, physical function and last but not least, fracture risk/incidence, may be further studied. Also, the association between fractures and milk consumption, as well as the association with specific dietary patterns.

The AGES-Reykjavik Study was designed to examine risk factors, including genetic susceptibility and gene/environment interaction, in relation to disease and disability in old age. As it is known that the active form of vitamin D takes part in regulating expression of several genes associated with bone and mineral homeostasis [151] it would be of interest to assess the possible association between serum 25(OH)D concentration, BMD, food intake, including milk and cod liver oil, and genetic make-up.

Furthermore, the significance of cod liver oil intake in childhood and adolescence for bone health warrants further study. Taking into account factors such as total intake of both vitamin A and D, as well as physical activity and sunlight exposure, might help elucidate the association between intake and BMD in early life.

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Papers I-IV

I

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VALIDITY OF RETROSPECTIVE DIET HISTORY: ASSESSING RECALL OF MIDLIFE DIET USING FOOD FREQUENCY QUESTIONNAIRE IN LATER LIFE

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Abstract: *Objectives:* Limited information exists on the validity of dietary information given by elderly people on their past diet. Here we test the relative validity of a food frequency questionnaire asking older persons about their midlife diet. *Design:* Retrospective food intake of 56-72-year-old subjects was estimated using a food frequency questionnaire designed for the AGES-Reykjavik Study (AGES-FFQ), an epidemiological study of older individuals. Results were compared with detailed dietary data gathered from the same individuals 18-19 years previously, i.e., in midlife, as part of a national cohort. Spearman correlation and cross-classifications were used to assess the ability of the AGES-FFQ to rank subjects according to their intake. *Setting:* Nationwide, Iceland. *Participants:* Subjects, born 1937-1952 (n=174), who participated in the 1990 Icelandic National Dietary Survey. *Measurements:* Dietary intake, estimated by the AGES-FFQ (2008-2009), and dietary history obtained from the 1990 Icelandic National Dietary Survey as a reference method. *Results:* The strongest correlation between the AGES-FFQ and the reference method was found for cod liver oil, r=0.53, p<0.001 and r=0.56, p<0.001, for men and women, respectively. For men the corresponding correlation coefficient for milk and dairy products was r=0.43, p<0.001. The correlation coefficients were lower but within a reasonably acceptable range (r=0.26-0.40) for meat, fish and potatoes for both genders, as well as fresh fruits and milk/dairy products for women and whole-wheat bread, oatmeal/muesli and blood/liver-sausage for men. No correlation was found between the AGES-FFQ and the dietary history for rye bread and vegetable consumption. Subjects were categorized into five groups according to level of consumption by the two methods. Cross-classification showed that 16-59% were classified into same group and 43-91% into same or adjacent group, 0-14% were grossly misclassified into opposite groups. *Conclusion:* The AGES-FFQ on midlife diet was found suitable to rank individuals by their intake of several important food groups.

Key words: Food frequency questionnaire, validity, midlife diet, elderly.

Introduction

With aging populations, demand on healthcare and social service systems is expected to increase, due to higher prevalence of chronic and degenerative diseases among older age groups (1-3). Investing in healthy ageing and studying determinants of health in older age groups can therefore have significant advantages for society as well as the aged.

Various environmental factors can affect health in late life (4), food habits and dietary intake being among the strongest (1, 4). As most lifestyle-related diseases develop over long periods before being detected (5), successful aging may partially be determined by eating habits and dietary choices made decades earlier (6, 7).

Studies on the relationship between diet earlier in life and healthy aging are of special interest in this regard. However, few methods studies have been able to examine information on remote intake from dietary studies of elderly cohorts, making the use of recalled diets from years back an interesting field of research. Information is limited on the validity of elderly people's answers when asked about remote diet. Studies suggest that past diet may be recalled with acceptable accuracy up to 10 years prior, though greater uncertainty exists beyond this period (5, 6, 8-10). Various factors influence our ability to recall past diet. Even though time is an important factor, frequency and pattern of consumption of individual foods may be even more important, and may affect the way people remember and report past diet (11).

A good way to assess the validity of remotely recalled diet is resurveying individuals who have provided detailed dietary information in the past. Two different dietary assessment methods should ideally be used, the former being a detailed one, while the latter should be suitable for epidemiological studies, such as a food frequency questionnaire (5).

The food frequency questionnaire being assessed here is used in the Age/Gene Environment Susceptibility - Reykjavik Study (AGES-Reykjavik), conducted by the Icelandic Heart Association and the NIA (National Institute on Aging) Intramural Research Program. The study, initiated in 2002, includes 5,764 participants born 1907-1935. It was designed to examine risk factors, such as genetic susceptibility and gene/environment interaction, including diet, in relation to

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disease and disability in old age. Extensive health-related variables have been gathered for all participants, along with information on lifestyle and food intake (AGES-FFQ). The study was approved by the Icelandic National Bioethics Committee (VSN: 00-063) (12) and the MedStar IRB for the Intramural Research Program, Baltimore, MD.

In the present study, a detailed dietary history gathered 18-19 years previously presented a unique opportunity to test the validity of questions on remote diet. The objective of the study was to test the relative validity, i.e. the ability of the questions to acceptably rank individuals according to intake. Assessing the validity of the AGES-FFQ is essential for studying the relationship between health and diet at different periods of life in this large study, here focusing on the middle-aged (40-50y). Furthermore, it may shed important light on the limitations and use of recalled diet in epidemiologic studies of older individuals.

Subjects and methods

Subjects

The subjects were eligible participants in the Icelandic National Dietary Survey (INDS) conducted in 1990, for which detailed dietary information exists, gathered 18-19 years before the present study. The sample from 1990 included 1725 individuals, aged 15-80, selected randomly from the national registry of that time. The participation rate was 72%.

The present study aimed at assessing whether the AGES-Reykjavik food frequency questionnaire could acceptably rank individuals according to dietary intake in midlife (approximately 40-50 years of age). We therefore chose to include only participants who were 38-53 years of age at the time of the 1990 INDS. This slightly wider age bracket was chosen to enlarge the sample size (to approximately n=300) and increase the study's power.

Altogether 326 individuals were in the original sample; thereof fifteen individuals were deceased, and six had moved abroad. An invitation letter and the AGES-FFQ were sent to the 305 eligible participants, 167 women and 138 men, in October-December 2008. Of these, 174 returned completed questionnaires (57%), 107 women and 67 men. Their average age at the time of the 1990 survey was 44 (±4.6y).

Methods

The validity of the AGES-FFQ was assessed by comparing the answers to the questions on remote diet to the dietary data from the 1990 INDS.

The 1990 INDS gathered dietary information with a detailed dietary history focusing on the last three months. Each participant met with an interviewer in an hour-long interview taken in the participant's home, work place or a local clinic. The interviewers (n=32) were teachers or students of nutrition or food sciences. They took a ten-day course prior to the survey for training and synchronizing methods. The participants answered questions on usual diet as well as on socioeconomic factors and personal issues, i.e., age, height, weight, smoking habits, exercise, employment, working hours, education, family type and income. When assessing usual diet, the interviewer asked about each meal/snack of the day, which food groups/items were usually consumed, how often and in what quantities, also which cooking methods were commonly used (baked/boiled/fried, etc.). Quantities were estimated using photographs of different portion sizes, as well as measurement glasses and bowls. Consumption was recorded as times per month, and daily intake of food (grams per day) calculated accordingly (13).

The food frequency questionnaire

The food frequency questionnaire was developed for the AGES study and contains a total of 17 questions on midlife diet (40-50y). The questions ask the average frequency of intake of general food groups, e.g., milk and dairy, fish, meat, bread, fruits and vegetables. Foods and food groups were selected for the questionnaire on the basis of their importance in Icelanders' diet, according to former National Nutrition Surveys, or based on their unique nutritional qualities. Figure1 shows an example question from the AGES-FFQ. The AGES-FFQ was used to rank individuals according to level of intake of general food groups.

Statistical analysis

Statistical analysis was done using the computer program SPSS version 11.0. Kolmogorov-Smirnov tests for normal distribution of data showed that the distribution of the data from the AGES-FFQ was not normal; neither was most of the 1990 data for intake of food groups.

In assessing how well the participants represented the study sample in relation to average dietary intake, student t-tests or Mann-Whitney U tests were performed depending on distribution of data.

To assess correlation between the 1990 data and the answers from the AGES-FFQ, the 1990 data was transformed into times per week, agreeing with the classification of the answers from the AGES-FFQ. Predetermined portion sizes were used, taking into consideration both average daily consumption from the 1990 INDS and recommended portion sizes from the Public Health Institute of Iceland (Table 2). The Spearman's rank correlation test was used to assess correlation. The correlation gives an idea of the validity of the FFQ.

A question was considered to reasonably rank individuals according to their intake of food groups when correlation between the two methods was ≥0.25. For nutrients the correlation coefficient >0.3 has been suggested to be satisfactory when validating present diet (5, 14). Also, validation studies have suggested a correlation of ≥0.4, or even 0.5-0.7 to be optimal (5, 15) when comparing measurements of food intake; however, these studies have been on present diet. Jia et al. (16) validated a food frequency questionnaire on present diet in free-living older people and found a correlation coefficient of ≥0.2 to be reasonably valid. Taking these points into consideration, the correlation coefficient of ≥0.25 ($p<0.05$) was considered acceptable in the present study, as the aim was to assess the validity of past diet. The number of participants was within the reference sample range for validation studies (100-200 persons), as found by Willet (5).

As the distribution of reported intake was skewed in the AGES-FFQ, it was not possible to divide subjects into quartiles or quintiles. For that reason the two lowest possible answers of the AGES-FFQ were combined, as well as the two highest ones. Answers to the questionnaire were then given values 1-5 according to recorded level of intake, level 1 being the lowest intake, never or less than once a week; level 2 equalling 1-2 times per week; level 3 equalling 3-4 times per week; level 4 equalling 5-6 times per week and level 5 being the highest, equalling daily or more than once per day. Data from the 1990 INDS was also transformed into categories agreeing with the 1-5 classification of the AGES-FFQ answers.

Cross-classification was then used to compare answers to the AGES-FFQ and data from the 1990 INDS to see the proportion of individuals falling into the same, adjacent or opposite category according to intake in the two surveys (using levels 1-5).

Results

Average food intake in 1990 was compared between the participants and the whole sample. No significant difference was found between average intake reported in the 1990 INDS of the whole sample (n=305), and intake of the 174 participants (Table 1).

Table 1
Comparison of consumption in the 1990 survey between whole sample and final participants. Consumption shown as median consumption along with 10th and 90th percentile

	Sample (n 305)			Participants (n 174)			
	P10	median	P90	P10	median	P90	p-value
Men							
Meat	62	145	278	57	138	249	0.476
Fish	30	85	160	21	89	155	0.823
Potato	63	150	286	58	146	314	0.889
Fruit	0	18	155	0	14	145	0.735
Blood-/liver sausage	0	0	43	0	0	45	0.514
Rye bread/flatbread	0	0	36	0	0	33	0.968
Whole-wheat	21	76	161	27	78	169	0.367
Oatmeal/muesli	0	0	96	0	0	97	0.733
Vegetable	7	41	110	8	33	111	0.521
Milk and dairy	113	485	1007	112	509	1022	0.748
Fish liver oil	0	0	13	0	0	13	0.442
Women							
Meat	30	82	146	41	85	147	0.593
Fish	27	54	97	28	57	96	0.878
Potato	30	91	180	29	92	183	0.854
Fruit	0	55	184	0	69	215	0.120
Blood-/liver sausage	0	0	28	0	5	30	0.263
Rye bread/flatbread	0	1	32	0	3	35	0.674
Whole-wheat	19	60	108	19	60	109	0.786
Oatmeal/muesli	0	0	53	0	0	75	0.495
Vegetable	12	55	135	18	64	163	0.302
Milk and dairy	74	341	662	74	336	677	0.861
Fish liver oil	0	0	13	0	0	13	0.150

In table 2, average daily intake in the 1990 INDS is compared with reported intake from the AGES-FFQ. Data is shown separately for men and women. The frequency of intake is similar between men and women according to the AGES-FFQ, but average consumption in grams from the 1990 INDS was different between the genders. As previously reported, the main characteristics of the Icelandic diet in 1990 were high intake of animal-based products, such as fish, meat, milk and dairy products, as well as relatively low intake of fruits, vegetables and cereals, especially cereals rich in fibre (13).

Validity of eleven of the seventeen questions in the AGES-FFQ was tested. Questions about certain foods could not be tested as information from the two methods could not be compared; consumption was either too low or sporadic, or the questions were not directly on frequency of intake. Six questions could not be tested for these reasons, i.e., consumption of fish bread toppings and salted/smoked fish and meat, type of milk commonly used, type of butter/margarine commonly used and amount of butter/margarine used per slice of bread.

Five of the eleven questions tested were found to be acceptable for ranking individuals according to level of food intake. Slight variation in results was found between genders. The strongest correlation was found for cod liver oil for both genders and milk and dairy products for men ($r=0.43$-0.56). The correlation coefficient was lower ($r=0.26$-0.35) for meat, fish and potatoes for both genders, and milk and dairy products for women. Furthermore, the question on fresh fruit consumption was found to be reasonably acceptable among women ($r=0.31$; $p=0.001$) but not men ($r=0.18$; $p=0.168$), and the questions on whole-wheat bread, oatmeal/muesli and blood/liver sausage were found to be reasonably acceptable among men ($r=0.40$; $p=0.001$; $r=0.28$; $p=0.026$; and $r=0.34$; $p=0.007$, respectively) but not women ($r=0.05$; $p=0.582$; $r=0.22$; $p=0.027$ and $r= 0.21$; $p=0.029$, respectively). The questions on rye bread and vegetable consumption were not valid for either gender.

Data from the 1990 INDS was transformed into categories agreeing with the 1-5 classification of the AGES-FFQ answers to see the proportions of subjects falling into the same groups by the two methods using cross-classification (Table 3).

The percentage of participants classified into the same group was 16-59% (average 37%) and 43-91% (average 69%) into the same or adjacent group. Between 0-14% (average 4%) were grossly misclassified into the opposite group. Individuals were most likely to be classified into same/adjacent category in relation to their fish and blood/liver sausage consumption and most likely to be grossly misclassified in relation to their vegetable and cod liver oil consumption.

Table 2
Consumption of participants in the 1990 survey compared to reported consumption according to the AGES-FFQ

	1990 g/d				1990 times per week			FFQ times per week				
	P10	**median**	**P90**	**g per portion**	**P10**	**median**	**P90**	**P10**	**median**	**P90**	**correlation**	**p-value**
Men												
Meat	57	138	249	150	2.6	6.5	12.3	1.5	3.5	5.5	0.298	0.016
Fish	21	89	155	150	1.1	3.8	7.8	1.5	3.5	3.5	0.260	0.037
Potato	58	146	314	120	3.4	8.5	18.3	4.3	7.0	7.0	0.348	0.005
Fruit	0	14	145	100	0	0.8	9.8	0.3	1.5	4.7	0.176	0.168
Blood-/liver sausage	0	0	45	70	0	0	4.5	0.3	0.3	1.5	0.335	0.007
Rye bread/flatbread	0	0	33	40	0	0	5.8	0.3	1.5	5.5	0.154	0.224
Whole-wheat bread	27	78	169	54	3.5	10	22.0	3.5	5.5	7.0	0.395	0.001
Oatmeal/muesli	0	0	97	100	0	0	6.8	0	0.3	7.0	0.277	0.026
Vegetable	8	33	111	100	0.6	2.3	7.8	0.3	1.5	5.5	0.153	0.222
Milk and dairy	112	509	1022	400	1.8	8.8	18.5	1.5	7.0	14.0	0.432	0.000
Cod liver oil	0	0	13	13	0	0.3	7.0	0	3.5	7.0	0.534	0.000
Women												
Meat	41	85	147	150	1.8	3.7	6.7	1.5	3.5	5.5	0.255	0.009
Fish	28	57	96	150	1.1	2.3	4.4	1.5	3.5	3.5	0.281	0.004
Potato	29	92	183	120	1.7	5.4	10.7	3.5	7.0	7.0	0.259	0.008
Fruit	0	69	215	100	0	5.0	15.1	0.3	3.5	7.0	0.313	0.001
Blood-/liver sausage	0	5	30	70	0	0.5	3.0	0.3	0.3	1.5	0.214	0.029
Rye bread/flatbread	0	3	35	40	0	0.5	6.1	0.3	3.5	7.0	0.066	0.507
Whole-wheat bread	19	60	109	54	2.5	7.8	14.2	3.5	7.0	7.0	0.054	0.582
Oatmeal/muesli	0	0	75	100	0	0	5.3	0	1.5	7.0	0.215	0.027
Vegetable	18	64	163	100	1.2	4.5	11.4	0.3	3.5	7.0	0.079	0.418
Milk and dairy	74	336	677	400	1.1	5.8	12.0	0.3	7.0	7.0	0.290	0.003
Cod liver oil	0	0	13	13	0	0	7.0	0	3.5	7.0	0.563	0.000

Data from the 1990 survey in grams/day and transformed into times per week using predetermined portion sizes. Data from the AGES-FFQ shown as times per week

Table 3
Cross-classification of participants, portion in same/adjacent group or grossly misclassified

	Same category N (%)		Same or adjacent category N (%)		Grossly misclassified N (%)	
	Men	**Women**	**Men**	**Women**	**Men**	**Women**
Meat as a main meal	13 (20.0)	42 (40.8)	35 (53.9)	88 (85.4)	1 (1.5)	1 (1.0)
Fish as a main meal	21 (32.3)	51 (48.6)	51 (78.5)	96 (91.4)	0 (0)	1 (1.0)
Potatoes	37 (58.7)	37 (35.6)	51 (81.0)	73 (70.2)	1 (1.6)	1 (1.0)
Fruits	20 (31.7)	23 (22.8)	41 (65.1)	57 (56.4)	4 (6.3)	4 (4.0)
Blood/liver sausage	39 (60.9)	53 (51.0)	53 (82.8)	93 (89.4)	2 (3.1)	2 (1.9)
Rye bread	10 (15.6)	23 (22.3)	40 (62.5)	56 (54.4)	1 (1.6)	8 (7.8)
Whole-wheat bread	31 (47.7)	50 (47.6)	53 (81.5)	76 (72.4)	2 (3.1)	2 (1.9)
Oatmeal/muesli	32 (49.2)	44 (41.9)	43 (66.2)	72 (68.6)	6 (9.2)	11 (10.5)
Vegetables	12 (18.5)	21 (19.8)	34 (52.3)	46 (43.4)	5 (7.7)	9 (8.5)
Milk/dairy products	28 (43.1)	33 (32.0)	47 (72.3)	64 (62.1)	1 (1.5)	5 (4.9)
Cod liver oil	31 (50.0)	48 (46.6)	40 (64.5)	63 (61.2)	8 (12.9)	14 (13.6)

Discussion

Information is limited on the reliability of elderly people's answers to questions about their diet years or even decades earlier. In the present study we used detailed data from 1990 on the diet of middle-aged people to assess the validity of a questionnaire on remote diet. The frequency of intake of different food items, reported by the AGES-FFQ was found to correlate most strongly with the reference method for cod liver oil and milk and dairy products for men. The correlation coefficients were lower, though within an acceptable range, for meat, fish and potatoes for both genders, as well as fresh fruits and milk and dairy products for women, and whole-wheat bread, oatmeal/muesli and blood/liver-sausage for men. The questions on rye bread and vegetable consumption were not found to be acceptable for either gender.

Even though the consumption of fruits and vegetables among Icelanders has increased in recent decades, the consumption is still low and far from reaching recommended levels of ≥500g/d (17, 18). When compared to other Northern/European countries, Icelanders' consumption is especially low (18-20). In the present study, the question on vegetable consumption was not found to be valid; neither was the question on fresh fruits for men. A plausible explanation might be the low intake and limited distribution of intake in this age group. An alternative hypothesis is that the extreme weight difference between individual vegetable types, such as leafy vegetables versus tubers, may contribute to the observed lack of relationship between the frequency of consumption and grams per day of this food group.

Figure 1
Example question from the AGES-FFQ

32. On average, how often did you eat fish or a fish dish for a main meal when you were middle aged (40-50y)?

- ❒ Never
- ❒ Less than once a week
- ❒ 1-2 times a week
- ❒ 3-4 times a week
- ❒ 5-6 times a week
- ❒ Daily
- ❒ More than once a day

However, validating questions on fruit and vegetable intake as part of Icelanders' present diet has yielded better results, in studies of both adults and children (21, 22).

The frequency and pattern of consumption seem to greatly affect how people remember past diet. Food consumed daily/several times per day (e.g., milk) may be reported more accurately than foods consumed sporadically/several times per month/week (e.g., poultry). Conversely, food consumed quite rarely, e.g., only on holidays, can also be remembered and reported fairly accurately (11). The present study verified these factors, formerly pointed out by Dwyer and Coleman. Both studies underline the importance of validation measures and studies for retrospectively collected intake data on food groups and special food items. The present study found that consumption of milk and dairy products, potatoes and cod liver oil (foods often consumed daily) was generally more accurately reported than, e.g., fresh fruits and vegetable, eaten several times per week on average. Inconsistency was also found in the reported intake of bread, especially rye bread, which is much less common in the Icelandic diet than wheat bread. Dwyer and Coleman (11) also stated that consumption of dietary supplements with special characteristics (such as cod liver oil) might be recalled especially well, which was also the case in the present study.

Reported consumption of cod liver oil had the strongest correlation to reported actual intake from 1990 (r>0.5 for both genders). The reason is perhaps that cod liver oil was generally consumed either every day or not at all; very few subjects consumed cod liver oil sporadically. Interestingly, cod liver oil was also most frequently grossly misclassified, as approximately 13% of participants were classified into the opposite category when comparing the AGES-FFQ to the 1990 data, subjects most commonly reporting daily intake according to the AGES-FFQ, while the data from 1990 reveal no intake. A likely explanation for this seeming discrepancy is that cod liver oil is taken seasonally by many Icelanders, to provide vitamin D during the dark winter months. The reference dietary history method from 1990 was designed to reflect the diet during the past 3 months, while the AGES-FFQ should reflect the usual diet during a whole decade. Therefore, some individuals interviewed in the summer of 1990 may not have been taking cod liver oil at that time, although it may have been a part of their daily diet for most of the year.

The AGES-FFQ on midlife diet was found to quite well represent intake of cod liver oil and thereby intake of vitamin D. The northern latitude of Iceland results in limited production of vitamin D3 during wintertime (23) and consequently very low 25(OH)D levels in adults not taking vitamin D supplements (24, 25). Cod liver oil is traditionally used in Iceland as a vitamin-D source, and the dietary intake of vitamin D from other sources is very limited. The acceptable validity of the AGES-FFQ regarding cod liver oil consumption makes it possible to estimate association between the consumption of cod liver oil and vitamin D intake with several important health-related endpoints.

It should be noted that the correlation coefficient of 0.25 was considered reasonably acceptable in the present study. Although the results show that the AGES-FFQ on remote diet is able to rank individuals according to their intake of several important food groups, one should always be aware of the limitations of the method and the different results seen for different food items. The present study highlights the importance of testing the validity of questions on different food groups, and assessing each question in the questionnaire separately, as validity might differ greatly between questions.

Due to the perception that healthy aging relies to some extent on a healthy and well-balanced diet in earlier life, the ability to recall remote diet is of great interest. With better understanding of the factors most strongly influencing the development of various diseases and having the strongest relations to health and quality of life in the elderly, special preventive measures could be promoted or strengthened and the likelihood of healthy ageing enhanced.

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Assessing validity of a short food frequency questionnaire on present dietary intake of elderly Icelanders

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Abstract

Background: Few studies exist on the validity of food frequency questionnaires (FFQs) administered to elderly people. The aim of this study was to assess the validity of a short FFQ on present dietary intake, developed specially for the AGES-Reykjavik Study, which includes 5,764 elderly individuals. Assessing the validity of FFQs is essential before they are used in studies on diet-related disease risk and health outcomes.

Method: 128 healthy elderly participants (74 y ± 5.7; 58.6% female) answered the AGES-FFQ, and subsequently filled out a 3-day weighed food record. Validity of the AGES-FFQ was assessed by comparing its answers to the dietary data obtained from the weighed food records, using Spearman's rank correlation, Chi-Square/Kendall's tau, and a Jonckheere-Terpstra test for trend.

Result: For men a correlation ≥ 0.4 was found for potatoes, fresh fruits, oatmeal/muesli, cakes/cookies, candy, dairy products, milk, pure fruit juice, cod liver oil, coffee, tea and sugar in coffee/tea (r = 0.40-0.71). A lower, but acceptable, correlation was also found for raw vegetables (r = 0.33). The highest correlation for women was found for consumption of rye bread, oatmeal/muesli, raw vegetables, candy, dairy products, milk, pure fruit juice, cod liver oil, coffee and tea (r = 0.40-0.61). An acceptable correlation was also found for fish topping/salad, fresh fruit, blood/liver sausage, whole-wheat bread, and sugar in coffee/tea (r = 0.28-0.37). Questions on meat/fish meals, cooked vegetables and soft drinks did not show a significant correlation to the reference method. Pearson Chi-Square and Kendall's tau showed similar results, as did the Jonckheere-Terpstra trend test.

Conclusion: A majority of the questions in the AGES-FFQ had an acceptable correlation and may be used to rank individuals according to their level of intake of several important foods/food groups. The AGES-FFQ on present diet may therefore be used to study the relationship between consumption of several specific foods/food groups and various health-related endpoints gathered in the AGES-Reykjavik Study.

Keywords: Food frequency questionnaire, Validity, Elderly, Nutrition

Background

Food frequency questionnaires (FFQ) are important research tools in nutritional epidemiology, and assessing their validity is an essential prerequisite for their use in studies of diet-related disease risk [1,2]. Few studies exist on the validity of FFQs administered to elderly people [3-5], and many of the instruments used were originally developed for younger subjects. Hence, their reliability and validity when administered to older subjects is uncertain [6,7].

It is always a challenge to assess dietary intake, and perhaps even more so when elderly individuals are concerned. Various factors related to older age, such as fading memory, declined cognitive function, and impaired hearing and/or vision may possibly affect the ability to give reliable information on dietary intake [4-10]. It has been suggested that FFQs may be a more appropriate assessment method for older people than, for example, 24 hour recalls [5,6] as older individuals may have more problems with short-term than long-term recalls, as well as more difficulties

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with open-ended recalls than with structured ones [10]. The length of interviews and questionnaires is crucial as older people may require longer to answer and may become more fatigued and frustrated than younger people [5]. Long and extensive FFQs may therefore contribute to lower response rates among elderly people [4].

Weighed food records are widely used and accepted as an appropriate reference method when validating FFQs [11,12]. In spite of inherent weaknesses of any dietary assessment method, food records have often been considered as the "gold standard" as they can provide relatively accurate quantitative information on consumption. Elderly participants have proved to be capable of keeping food records with acceptable levels of compliance and completion [13], and food records have been found to provide valid intake data for free-living elderly individuals [14]. Generally, a food record consisting of 3-4 consecutive days is recommended, as studies have shown that incomplete records get more frequent as the number of days increases. This is referred to as respondent fatigue [15,16].

While short FFQs lack the detail of longer questionnaires or food records, they have nevertheless been found to adequately assess the intake of specific foods and rank individuals with respect to selected nutrients [17-20]. The short food frequency questionnaire (AGES-FFQ) being assessed here was specially designed for the AGES-Reykjavik study, with 5,764 elderly participants. The AGES-Reykjavik study examines risk factors, genetic susceptibility, and gene/environment interaction, including diet, in relation to disease and disability in old age. Extensive health-related variables have been gathered for all participants. The AGES-Reykjavik study has been described previously [9]. The study was approved by the Icelandic National Bioethics Committee (VSN: 00-063) and the MedStar IRB for the Intramural Research Program, Baltimore, MD. The AGES-FFQ is threefold, including questions on diet in early life (14-19 y), midlife (40-50 y) and present diet. The validity of questions on midlife diet has been assessed in a previous study, where simple questions on consumption of, e.g., fish, meat, milk/dairy products, and cod liver oil were found to be valid [21].

The aim of this study is to assess the validity and ability of the AGES-FFQ to rank individuals according to intake of selected foods and food groups and to distinguish between individuals having high vs. low intake. Assessing the validity of the AGES-FFQ is essential before studying the relationship between present diet and health-related variables in the AGES-Reykjavik Study.

Methods

Subjects and setting

Subjects were healthy, elderly people, 65 years and older (58.6% female), and were a subsample of participants in the IceProQualita study, which focuses on the effects of training and food supplements on various health factors and health-related quality of life among the elderly [22]. Participants were recruited into the IceProQualita by advertisements posted in community centres and residential care homes in the capital area of Iceland. The advertisements included information on the study protocol and contact numbers. Willing and eligible individuals phoned in for further information and registration. A total of 284 individuals were registered and screened; 47 were excluded, leaving 237 participants at baseline. Exclusion criteria were cognitive function < 19 points on the MMSE [23], uncontrolled coronary heart disease, pharmacological interventions with exogenous testosterone or other drugs known to influence muscle mass, and major orthopaedic disease. Participant also had to be free of any musculoskeletal disorders, had to be weight stable and all women postmenopausal.

Our subsample consisted of the first 137 participants enrolled into the IceProQualita Study by March 2009, when data analysis for the present study began. By that time these individuals had undergone all baseline measurements, filled out a 3-day food record, the AGES-FFQ on present diet, and signed an informed written consent. The IceProQualita study was approved by the Icelandic National Bioethics Committee (VSNb2008060007/03-15). Dietary records from nine individuals were considered incomplete or inadequate and were therefore excluded. Data from 128 participants were therefore included in this study. The dropout rate from the IceProQualita study was 12% (n = 29), illness and falls being the most common reason [22]. The dropout did not affect participation in the present study, however, as all validation data were gathered at baseline.

Furthermore, our subsample did not differ from the whole study group of the IceProQualita study regarding age, anthropometric measurements, physical performance test, and outcome of various questionnaires on, e.g., general health, anxiety, quality of life, and the Mini Mental State Examination (MMSE).

Design

All participants answered the AGES-FFQ and subsequently filled out a 3-day weighed food record within approximately two weeks. Participants also completed questionnaires on physical activity, health-related quality of life, and drug, vitamin and herbal medicine intake. Anthropometric measures were performed; body weight was measured in light underwear on a calibrated scale (model no. 708, Seca, Hamburg, Germany), and height was measured with a calibrated stadiometer (model no. 206; Seca, Hamburg, Germany) [22].

The AGES-FFQ was used to assess frequency of consumption of different foods and food groups in order to rank individuals according to their level of intake. Validity of the AGES-FFQ on present diet was assessed by comparing its answers to the dietary data obtained from the weighed food records.

The weighed food record

Before filling out the food records each participant met with a researcher and was provided with a household scale (PHILIPS Essence HR 2393) and a structured booklet for recording his or her intake. Participants received detailed oral instructions on how to weigh and record their intake and were shown how to use the household scale. Written instructions were also incorporated in the food booklets along with contact information in case any questions arose during recording. Participants were asked to record in the booklet all food and beverages consumed for three consecutive days (Thursday-Saturday or Sunday-Tuesday), along with dates and times of meals. The importance of maintaining their regular diets and weighing and recording all food and drink consumed was emphasized.

The food frequency questionnaire

The food frequency questionnaire was designed specifically for the AGES-Reykjavik Study and is divided into three parts, containing questions on early life diet (14-19 y), midlife diet (40-50 y) and present diet. The part of the questionnaire on present diet includes 30 questions, 21 of which are assessed here. These are questions on the average frequency of intake of major food groups, e.g., milk and dairy products, meat, fish, bread, fruits and vegetables, as well as questions on selected foods, such as rye bread, blood/liver sausage, oat meal porridge and cod liver oil. Foods and food groups were selected for the questionnaire on the basis of their contribution to the absolute intake of elderly Icelanders according to former National Nutrition Surveys, as well as their unique nutritional qualities and possible connection to the development of various diseases in later life. The remaining nine questions, not assessed in the present study, are on the frequency of hot meals, type of milk and dairy products most commonly used, type and amount of bread spread commonly used, and finally there are four questions related to salt consumption (perception of saltiness, consumption of salted meat, salt fish, and added salt to prepared meals).

A majority of the questions have the same possible response categories as the questionnaire was designed to be simple and easily completed by elderly individuals (Figure 1 shows an example of question and response categories from the AGES-FFQ). However, questions on coffee, tea and sugar in coffee/tea differed in that they asked about daily frequency rather than weekly frequency of consumption. The questions not assessed here also had different response categories related to types of products, such as low fat vs. high fat, and salt perception.

On average, how often do you drink milk (whole milk, low-fat milk, skim milk, 1%-low-fat milk)?

- ☐ Never
- ☐ Less than once a week
- ☐ 1-2 times a week
- ☐ 2-4 times a week
- ☐ 5-6 times a week
- ☐ Daily
- ☐ More than once a day

Figure 1 Example of a question from the AGES-FFQ on present diet and response categories.

Data analysis

Nutritional analysis and data management Data on the participants' intake according to the 3-day weighed food records were entered into an interview-based nutrient calculation program, ICEFOOD, designed for the national dietary survey of The Icelandic Nutrition Council [24]. The amount of foods/food groups was calculated from 452 food recipes, which are based on 1148 food items from the National Nutritional Database, ISGEM.

Individual intake in grams per day for each food/food group was calculated from the food records. Gender-specific portions were estimated taking into account actual intake in grams and eating occasions from the food diaries, as well as predetermined portion sizes used in our previous validation study of questions on midlife diet (Additional file 1) [21]. The gender-specific portions were used to calculate intake in grams from the AGES-FFQ. The correlation was calculated between grams of food intake according to the two methods, food records and the AGES-FFQ.

Statistical analysis Data were entered into the statistical package SPSS, version 11.0. Kolmogorov-Smirnov tests were used to test the distribution of data. The answers from the AGES-FFQ were not normally distributed; neither were most of the data from the food records.

Simple descriptive statistics were used to describe general characteristics of the study group and the AGES participants. To assess differences between groups, student t-tests, Mann- Whitney U-test and Chi-square test were used. Correlation between intake according to the

AGES-FFQ and the food records was assessed using Spearman's rank correlation. As the distribution of reported intake from the AGES-FFQ on present diet was skewed, subjects could not be divided into quartiles or quintiles. Data were therefore split into 2-4 groups, depending on the distribution of answers from each question of the AGES-FFQ. Kendall's tau-b rank correlation coefficient and Chi-Square tests were used to further examine association between the two methods.

Additionally, the computer program SAS version 9.1 was used to perform a nonparametric Jonckheere-Terpstra test for trend, to test if the categories according to the AGES-FFQ ranked mean intake from the food record in an anticipated, graded order.

The significance level was set at $p \leq 0.05$.

Results

Comparing the AGES-FFQ to the reference method (Table 1), a correlation ≥ 0.4 was found for potatoes, fresh fruits, oatmeal/muesli, cakes/cookies, candy, dairy products, milk, pure fruit juice, cod liver oil, coffee, tea and sugar in coffee/tea (r = 0.40-0.71) for men. Furthermore, a correlation of 0.33 was found for raw vegetables. For women, correlation ≥ 0.4 was found for rye bread, oatmeal/muesli, raw vegetables, candy, dairy products, milk, pure fruit juice, coffee, tea and cod liver oil (r = 0.40-0.61). A correlation between 0.3 and 0.37 was found for fish topping/salad, fresh fruit, blood/liver sausage, and sugar in coffee/tea. The correlation for whole-wheat bread was lower, but still significant (r = 0.28, $p = 0.017$). Questions on meat and fish consumption, as well as questions on cooked vegetables and soft drinks, were not found to have a significant correlation to the reference method.

The Jonckheere-Terpstra trend test gave comparable results to the Spearman's rank correlation, with the exception of fish topping/salad for men and cooked vegetables for women, which showed significant trend in spite of insignificant correlation (Additional file 2).

The Pearson Chi-Square and Kendall's tau gave similar results, also showing significant association for fish topping for men; however, no association was detected between the methods for consumption of raw vegetables and candy for men. Results for women were all comparable to the Spearman's rank correlation (Additional file 3).

General characteristics of participants in the present study are shown in Table 2, along with characteristics of the participants from the AGES-Reykjavik study, for which the AGES-FFQ was designed. Participants in the present study were on average slightly taller and significantly heavier, had a higher body mass index (BMI) and percent body fat than participants in AGES, and were on average 2.7 years younger than AGES participants.

Discussion

The present study was conducted to assess validity of a dietary questionnaire and test its ability to rank individuals according to the level of intake of specific foods and food groups.

It has been suggested that when validating a questionnaire on present diet using a reference method, correlation coefficients should be ≥ 0.3 preferably over 0.4 and optimally in the range of 0.5-0.7 [11,12,25]. Of the 21 questions assessed here, 13 questions for the men and 14 for the women had a correlation ≥ 0.3 thereof 12 questions for the men and 10 for the women had a correlation ≥ 0.4 The foods showing the highest correlation were not in all cases identical for both genders, and men generally had higher correlations than women. The questions that had a correlation above 0.3 for both genders were on fresh fruits, oatmeal/muesli, raw vegetables, candy, dairy products, milk, pure fruit juice, cod liver oil, coffee, tea, and sugar used in coffee/tea.

The correlation between the AGES-FFQ and the reference method was not significant for fish, meat, cooked vegetables and soft drinks/sweetened juices. Part of the explanation for low or no correlation in general may be the inability of a 3-day food record to adequately reflect individual intake of foods that are consumed infrequently. Soft drinks are an example of this possible limitation of the reference method. For such food the 3-day food record may not be the ideal reference method, as the food in question may not show up on the food record. The AGES-FFQ data might even be closer to true intake in these cases. However, fish, meat and cooked vegetables were not consumed infrequently (2-4 times per week on average), and food items less frequently consumed had acceptable correlation between the two methods. A possible explanation for no correlation for meat and fish consumption might be the lack of distribution for answers to the AGES-FFQ, as almost 90% of participants marked either of two options - 1-2 times a week or 3-4 times a week - reflecting the uniform consumption of both fish and meat in this age group. Answers to the question on cooked vegetables were slightly better distributed even though almost 70% of participants answered either of the two previously mentioned options. In such cases, results from the food records may be better suited to rank individuals' intake. The validity of global questions with narrow distribution of answers, such as for meat, fish and vegetables, could presumably be improved by increasing frequency options to improve distribution, as well as by splitting them up into separate questions on types of meat, fish, etc. It is known that global questions may underestimate consumption [26], and affect validity. Global questions, chosen for the sake of simplicity, may thus limit the validity of the AGES-FFQ.

Table 1 Correlation between grams of intake from food records and calculated intake from the AGES-FFQ

	Food record g/d			AGES-FFQ g/d				
	P10	median	P90	P10	median	P90	correlation	p-value
Men (n = 53)								
Meat	7	90	248	43	100	100	0.21	0.124
Fish	20	77	190	36	85	114	0.23	0.098
Fish toppings	0	0	23	2	10	23	0.23	0.146
Potatoes	22	74	174	24	86	110	0.46	< 0.001
Fresh fruits	0	87	226	24	86	110	0.50	< 0.001
Blood/liver sausage	0	0	13	0	3	17	0.05	0.746
Rye bread/flatbread	0	17	59	2	25	50	0.17	0.219
Whole-wheat bread	11	45	73	11	50	50	0.19	0.169
Oatmeal/muesli	0	0	227	0	95	190	0.46	0.001
Cooked vegetables	0	29	113	3	19	60	0.17	0.221
Raw vegetables	0	33	150	3	19	90	0.33	0.015
Cakes and cookies	0	51	137	10	15	70	0.41	0.002
Candy	0	0	13	0	8	18	0.40	0.003
Dairy products	0	84	241	7	103	205	0.55	< 0.001
Milk	0	133	560	0	83	264	0.49	< 0.001
Pure fruit juice	0	0	192	0	34	160	0.50	< 0.001
Soft drink and sweet juice	0	0	134	0	12	231	0.19	0.177
Cod liver oil	0	0	8	0	6	6	0.51	< 0.001
Coffee*	87	357	793	105	735	1155	0.63	< 0.001
Tea*	0	0	200	0	110	330	0.71	< 0.001
Sugar in coffee/tea*	0	0	11	0	0	8	0.53	< 0.001
Women (n = 75)								
Meat	21	59	151	29	29	68	0.11	0.361
Fish	21	55	137	28	65	102	-0.02	0.873
Fish toppings	0	0	28	0	3	35	0.37	0.001
Potatoes	18	60	116	18	67	85	0.01	0.969
Fresh fruits	39	127	316	60	120	240	0.36	0.001
Blood/liver sausage	0	0	14	0	2	13	0.37	0.001
Rye bread/flatbread	0	7	40	2	30	60	0.42	< 0.001
Whole-wheat bread	1	35	76	10	45	45	0.28	0.017
Oatmeal/muesli	0	52	159	3	75	150	0.48	< 0.001
Cooked vegetables	0	25	90	3	45	90	0.20	0.089
Raw vegetables	0	53	141	17	40	80	0.40	< 0.001
Cakes and cookies	0	36	123	2	30	60	0.20	0.087
Candy	0	0	23	1	6	30	0.43	< 0.001
Dairy products	0	100	217	6	85	170	0.50	< 0.001
Milk	0	113	369	0	29	135	0.45	< 0.001
Pure fruit juice	0	0	167	0	34	160	0.49	< 0.001
Soft drink and sweet juice	0	0	148	0	8	48	0.19	0.104
Cod liver oil	0	2	9	0	7	7	0.42	< 0.001
Coffee*	67	283	647	98	293	683	0.44	< 0.001
Tea*	0	0	363	0	360	840	0.61	< 0.001
Sugar in coffee/tea*	0	0	3	0	0	0	0.30	0.008

* portions per day

Table 2 Comparison of study group and participants in the AGES-Reykjavik Study

	Men AGES	Study group	p-value	Women AGES	Study group	p-value
Participants, n	2102	53		2699	75	
Age, y (sd)	76.5 (5.3)	74.2 (6.0)	0.011	76.1 (5.5)	73.3 (5.5)	< 0.001
Height, cm (sd)	175.5 (6.2)	176.5 (7.1)	0.248	160.9 (5.7)	162.5 (5.7)	0.016
Weight, kg (sd)	82.6 (13.3)	92.7 (17.4)	< 0.001	70.5 (13.3)	74.8 (11.9)	0.004
Smokers, %	12	5.7	0.170	12.9	9.3	0.383
Physical activity, walk (sd)*	3.7 (3.0)	3.3 (4.0)	0.003	3.3 (3.7)	2.6 (2.6)	0.001
Abdominal circumference, cm (sd)	102.1 (10.5)	108.2 (12.7)	< 0.001	99.4 (12.9)	93.9 (11.2)	< 0.001
BMI, kg/m^2 (sd)	26.8 (3.8)	29.7 (4.9)	< 0.001	27.2 (4.8)	28.4 (4.6)	0.035
FFM, kg (sd)	63.8 (7.6)	56.7 (6.9)	< 0.001	45.8 (6.3)	41.1 (4.6)	< 0.001
FAT, kg (sd)	18.5 (7.0)	32.9 (11.0)	< 0.001	24.3 (7.4)	31.7 (8.9)	< 0.001
Percent body fat, % (sd)	21.8 (5.5)	34.5 (5.9)	< 0.001	34.0 (5.0)	41.9 (6.6)	< 0.001
Systolic blood pressure, mmHg (sd)	142.8 (19.8)	148.0 (19.6)	0.005	141.9 (20.6)	137.2 (16.8)	0.060
Diastolic blood pressure, mmHg (sd)	76.0 (9.4)	77.6 (9.6)	0.357	72.1 (9.5)	74.1 (8.9)	0.099

*Hours spent walking per week, average time over the whole year

BMI = Body mass index; FFM = Fat free mass; FAT = Fat mass

The results from the Jonckheere-Terpstra trend test, Kendall's tau-b and Pearson Chi-Square were mostly in agreement with the results from the Spearman's rank correlation, with few deviations. Limited and/or skewed distribution of answers from the AGES-FFQ may contribute to these differences between the methods.

The validity of questions on midlife diet (40-50 y) in the AGES-FFQ has previously been assessed [21]. Retrospective food intake was estimated, where elderly individuals answered the AGES-FFQ on midlife diet, and data were compared to a detailed dietary history, obtained from the same individuals 18-19 years previously, i.e., in midlife. Questions and frequency options were mostly similar for the two periods of life in the AGES-FFQ. In the validation study for midlife diet, the reference method may have been better able to detect correlation for food consumed < 2-3 times a week than the 3-day weighed food recording used in the present study. In the previous study a significant correlation was found for fish and meat consumption (r = 0.25-0.30), along with a stronger correlation for cod liver oil [21]. Part of the explanation for higher correlation in some cases might be linked to the detailed dietary history used as a reference method in the previous validation study, reflecting long-term diet. Another possible explanation for higher correlation for midlife diet might be that both dietary assessment methods, i.e., dietary history and FFQ, can be subject to similar sources of error, such as bias to overestimate foods considered healthy, and to underestimate foods considered unhealthy.

Looking at the distribution of intake according to the two methods, there was a tendency for higher consumption in grams from the food records than would be expected for certain foods/food groups, considering frequency of consumption according to the AGES-FFQ and the calculated consumption using gender-specific portions. This could partly be explained by exceptionally large portions consumed by a few individuals according to their food records. The largest single meat portion was 600 g; the largest portion of soda was 900 ml, and a few individuals had a daily consumption of milk ≥ 1000 ml, while their reported frequency of intake was 3-4 times per week to once a day. This discrepancy emphasizes the limitation of using an FFQ without portion sizes.

In an attempt to evaluate possible over-/underestimation of intake, frequency of intake was compared between the two methods, using actual eating occasions from the food records (data not shown). There was no clear sign of over-/underestimation related to gender or foods/food groups considered healthy/unhealthy. However, foods consumed infrequently according to the AGES-FFQ may not have shown up in the 3-day food records and lead to the perception of overestimation according to the AGES-FFQ. Reported frequency of milk intake according to the AGES-FFQ was generally lower than according to food records. One possible explanation may be that milk used in coffee/tea, or milk poured on breakfast cereals/porridge was not included when answering the AGES-FFQ.

In order to evaluate the representativeness of our study group, general characteristics of the group were compared to the participants of the AGES-Reykjavik study, for which the AGES-FFQ was designed. The AGES-Reykjavik study originates from the Reykjavik study established in 1967, which consisted of 30,795 randomly sampled men and women born 1907-1935. This large cohort equalled roughly 35% of this age-specific population in Iceland. The AGES-Reykjavik cohort was randomly sampled from the 11,549 individuals still alive when examinations began and is thought to represent the study population fairly well. The participants in the present study were heavier, had less fat free (FFM) mass, more fat mass (FAT) and a higher BMI. The weight and amount of FAT may possibly be related to our study group being slightly younger than the average participant in the AGES study, as aging is commonly accompanied by weight loss [27,28]. With respect to the lower FFM of our participants, the fact that they signed up voluntarily to participate in the IceProQualita study, which included supervised exercise three times per week, may indicate that they themselves felt their physical fitness needed improvement, and that their weight should be better managed. This is further emphasized by the fact that our participants spent less time walking than the AGES participants, indicating that they were less physically active.

In spite of statistical significance for selected variables between participants of the present study and the AGES study, these differences are not extensive. Therefore, our study sample is still thought to represent the AGES group adequately for the purposes of validation.

Weighed food records are generally perceived as a good measure of food intake [11,12], and have the least correlated errors with food frequency questionnaires [12]. However, day-to-day variation can be great and even greater for individual food items than for nutrient intake [11]. Hence, a longer period of food recording, or repeated recordings, would have been needed in the present study to find correlation to certain answers of the AGES-FFQ.

Nonetheless, a majority of the questions in the AGES-FFQ had an acceptable correlation (r = 0.3-0.7) and may therefore be used to rank individuals according to intake. Questions with lower or insignificant correlation, such as on fish and meat consumption, should not be ruled out or considered invalid without further assessment, as the validity of certain questions is likely to be underestimated rather than exaggerated. However, the same applies here as in the previous study on the AGES-FFQ on midlife diet, that is, that even though the AGES-FFQ on present diet is able to rank individuals according to their intake of several important food groups, one should always be aware of the limitations of the method and the different results seen for different food items. It should also be noted that the AGES-FFQ is only appropriate for ranking individuals according to level of intake of selected foods and food groups, and not for assessing total food intake, energy or nutrients.

Conclusion

One of the most important factors related to health and quality of life in old age is nutrition [29-32]. It is also a factor we largely control ourselves and can therefore adjust to enhance our likelihood of successful aging [9,32,33]. Studies have shown that even in old age, adherence to a healthy diet or changes in lifestyle to improve health can affect risk factors for chronic diseases [34-36]. While some conditions develop over many years, others may occur within weeks [37].

It is our conclusion that the AGES-FFQ on present diet may be used to rank individuals according to consumption of several important foods and food groups. As a result the extensive data gathered from the elderly participants of the AGES-Reykjavik Study may be available for studies of associations between diet and health-related variables in this large epidemiological study.

Additional material

Additional file 1: Gender-specific portions (g) were estimated taking into account actual intake in grams and eating occasions from food diaries, as well as predetermined portion sizes used in a previous validation study of questions on midlife diet.

Additional file 2: Shows the results from the nonparametric Jonckheere-Terpstra test for trend performed to assess whether the AGES-FFQ ranked mean intake from the food record in an anticipated, graded order.

Additional file 3: Shows the results from Pearson Chi-Square and Kendall's tau-b tests performed to further assess the association between the two different dietary assessment methods.

Abbreviations

FFQ: Food Frequency Questionnaire; AGES (Reykjavik Study): Age Gene/Environment Susceptibility (Reykjavik Study); Y: year; MMSE: Mini Mental State Examination; BMI: Body mass index; FFM: Fat free mass; FAT: Fat mass.

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Authors' contributions

TE gathered dietary data, handled statistical analysis and interpretation of data, and drafted the manuscript. IT, IG and LS participated in the conception and design of the study and revision of the manuscript. LS contributed to the design of the food frequency questionnaire. All authors have read and approved the final manuscript.

Authors' information

TE is a PhD student and has assessed the validity of questions in the AGES-FFQ on midlife diet in a previous paper. LS, currently a professor of nutrition at the University of Iceland, was formerly director of the Icelandic Nutrition Council, and in that capacity managed two national nutrition surveys as well as other programs of the council, including co-operative projects with the Icelandic Heart Association. IT is a professor of nutrition and head of the Unit for Nutrition Research, University of Iceland and Landspitali National University Hospital. IT has long experience in co-ordinating studies e.g., validation studies, and is a principal investigator of the IceProqualita study. IG, a professor of nutrition at the University of Iceland, has co-ordinated and participated in several dietary surveys, some of them involving validation of methods. LS and IT supervise TE's PhD research project and IG is a member of TE's PhD committee.

Competing interests
The authors declare that they have no competing interests.

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III

Title page

Milk consumption throughout life is associated with bone mineral density in elderly men and women

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Abstract (150-250 words)

Purpose

The role of lifelong milk consumption for bone mineral density (BMD) in old age is not clear. Here we assess the association between hip BMD in old age and milk consumption in adolescence, midlife and current old age.

Methods

Participants of the AGES-Reykjavik Study, age 66-96 years (N=4798) reported retrospective milk intake during adolescence and midlife as well as in current old age, using a validated food frequency questionnaire. BMD of femoral neck and trochanteric area was measured by volumetric quantitative computed tomography (QCT). Association was assessed using linear regression models, and difference in BMD in relation to milk intake portrayed as gender specific Z-scores.

Results

Men with current milk consumption of ≥once/day compared with <once/week had Z-scores 0.9 higher on average for femoral neck (95% Confidence Interval -0.01,0.20). For midlife consumption the difference was 0.21 Z-scores (95% CI 0.04,0.38), and for consumption during adolescence the difference was 0.15 Z-scores (95% CI -0.16,0.45). Results were comparable for femoral neck and trochanter, and for men and women, though associations were generally slightly stronger for men.

Conclusions

Our data suggest that milk intake during midlife has the strongest association with hip BMD in old age, and is stronger for men than women.

Keywords (4-6 words)

AGES-Reykjavik Study, lifelong milk intake, old age bone mineral density

Introduction

Daily consumption of milk and dairy products is widely recommended for people of all ages [1-3], not the least for their high calcium content and importance for obtaining and maintaining bone health. Still the evidence is inconclusive when it comes to the significance of lifelong consumption of milk and dairy products for bone health in old age, the time when serious and debilitating osteoporotic fractures are most likely to occur [4].

While intervention studies have reported a positive effect of milk consumption in childhood and adolescence on bone mineralization and bone mineral density (BMD) [5-7], most long-term studies of calcium supplementation find the increase to be transient [8-10]. Conversely, cross-sectional and cohort studies have reported a positive association between milk intake in early life, both before and after puberty, and bone health in adulthood [11-16], as well as between midlife intake and bone health in old age [13,14]. Few studies are available on whole life exposure, from adolescence to old age, and long-term association between milk consumption and late life BMD, and results from epidemiological studies are still inconclusive on this point [13,14,16-19]. Furthermore, studies on the role of milk/dairy consumption on bone health of elderly men are lacking. More information is needed to better understand and assess the strength of this relationship and to identify the time periods most critical when it comes to bone health in old age.

The Age, Gene/Environment Susceptibility – Reykjavik Study (AGES-Reykjavik) is a study with 5,764 elderly participants (42% men). Extensive data on multiple lifestyle, health and biological factors have been gathered, including data on BMD at different skeletal sites, using quantitative computed tomography (QCT) scanning, and dietary intake at different periods of life (14-19y; 40-50y; current) using a validated food frequency questionnaire [20-22].

In this study our aims were to investigate whether, and to what extent, retrospective self-reports of milk/dairy intake in adolescence and midlife, as well as current old age, were associated with hip BMD in old age in participants of the AGES-Reykjavik Study. BMD of the hip was chosen over other skeletal sites due to the great impact hip fractures have on the elderly, their quality of life and life expectancy, as well as their substantial cost for the health care systems.

Methods and Subjects

Subjects - Design

The AGES-Reykjavik Study originates from the Reykjavik Study, a large population-based cohort study that started in 1967. All men and women born in 1907-1935 (n=30,795) and residing in Reykjavik and nearby communities in 1967 were selected, 27,281 were invited to participate and 19,381 attended [20, 23-25]. Of the 11,549 previously examined Reykjavik Study cohort members still alive when AGES-Reykjavik examinations began in 2002, 8,030 individuals were randomly chosen and invited to participate. From these, 5,764 individuals (42% male) had participated in the AGES-Reykjavik study by its conclusion in 2006. Participants were 66-96 years old at the time of examinations, average age being 76 years.

The AGES-Reykjavik examination was completed in three clinic visits within a 4- to 6-week time window. Extensive data were collected during clinical examinations, e.g. on physical and cognitive function, anthropometry, health history, and food history during adolescence, midlife and late life, i.e. current diet. Participants also underwent QCT-scanning and were asked to bring to the clinic all medications and supplements used in the previous two weeks, representing current usage [20,26].

Of the 5,764 participants of AGES-Reykjavik, 933 did not undergo the QCT scanning and additional 33 individuals did not give information on milk consumption in the AGES-FFQ, therefore data from 4,798 individuals (44% male) were used in the present study.

The AGES-Reykjavik Study was approved by the Icelandic National Bioethics Committee (VSN: 00-063) and the MedStar IRB for the Intramural Research Program, Baltimore, MD.

Bone measurements

QCT measurements, providing true volumetric density, were performed on the left hip using a 4-detector CT system (Sensation, Siemens Medical Systems, Erlangen Germany). Scans were acquired using a standardized protocol and encompassed the proximal femur from a level 1cm above the acetabulum to a level 5mm inferior to the lesser trochanter with 1mm slice thickness. Further procedures and quality assessments have been described in detail elsewhere [20,27].

The variables used in the present study are volumetric integral BMD (g/cc), reflecting both trabecular and cortical bone mass, of femoral neck and trochanteric area, encompassing both trochanters, as well as CT-derived simulated area of the femoral neck (cm^2). Reasons for exclusion from the QCT were inability to lie supine or weight over 150kg. Furthermore, hip scans were not performed on individuals that had undergone hip replacement surgery.

Dietary information

Dietary data were collected using a short food frequency questionnaire designed for participants of the AGES-Reykjavik study (AGES-FFQ). The questionnaire is divided into three parts, asking about intake at different periods of life; 16 questions on adolescent diet (14-19y), 17 questions on midlife diet (40-50y) and 30 questions on current diet. Foods and food groups were selected for the questionnaire on the basis of their contribution to the absolute intake of elderly Icelanders according to a National Nutrition Survey [28] as well as their unique nutritional qualities and possible connection to the development of various diseases in later life. The questionnaire has been described previously [21,22].

Frequency of consumption of milk and dairy products, excluding cheese, (hereafter referred to as milk) was measured in each of the three parts of the AGES-FFQ. The response categories were: 1) Never, 2) Less than once a week, 3) 1-2 times a week, 4) 3-4 times a week, 5) 5-6 times a week 6) Daily or 7) More than once a day.

Validity of questions on midlife diet and current diet has been assessed in previous studies, and milk was among the foods that showed the highest validity [21,22]. Midlife milk intake of 56-72-year old individuals was estimated retrospectively using the questions on midlife diet in the AGES-FFQ and results compared with detailed dietary data, gathered from the same individuals 18-19 years previously, i.e. in midlife, as a part of a national dietary survey [28]. Correlation using Spearman's rho was $r=0.43$, $p<0.001$; $r=0.29$, $p=0.003$ for men and women respectively [21]. Validity of current milk intake was assessed among elderly individuals (average age 74y) by comparing answers of the AGES-FFQ to 3-day weighed food records completed by the same individuals. Correlation using Spearman's rho was $r=0.49$, $p<0.001$; $r=0.45$, $p<0.001$ for men and women respectively [22].

Covariates

For examining the association between milk consumption through different periods of life and hip BMD we selected *a priori* the following set of covariates: age, physical activity during different periods of life, current alcohol intake, cod liver oil intake (main dietary source of vitamin D in the population studied) in the same time period as milk intake, and body mass index (BMI). Midlife BMI was chosen as a covariate for the retrospective data, and current BMI for current data.

To obtain history of physical activity, participants were asked how much time (hours/week) they spent on moderate to vigorous activities in four different periods of life; young adulthood (20-34y), early middle age (35-49y), late middle age (50-64y) and current physical activity. Both weight bearing and non-weight bearing exercises were included. Current consumption of alcohol was converted into grams per week using 14g of alcohol as a standard drink and then divided into <25g/week, 25-50g/week, and >50g/week. Information on cod liver oil intake was gathered using the AGES-FFQ [21,22]. Midlife data on BMI had been collected in the Reykjavik-Study [23].

For early life most of these covariates can only be considered surrogate measures of corresponding early life characteristics. On the other hand for midlife and in current old age covariates selected are potential predictors of both bone health and dietary habits.

Data analysis – Statistical analysis

Characteristics of study participants were described using mean and standard deviation of normal variables, median and interquartile range for skewed variables and percentages for dichotomous variables. Due to the approximate normal distribution of the source BMD variables they were transformed into sex-specific z-scores, reflecting the number of standard deviations (SD) from the mean BMD in our population of 66-96 years of age.

Univariate and multivariate linear regression was used for examining the association between milk intake and BMD. Milk consumption was categorized *a priori* into three groups: ≤ once/week, 1-6 times/week and ≥once/day. The lowest intake group (≤ once/week) was in all cases used as referent and results are represented as difference (Δ) in z-score with increased frequency of consumption compared to the referent. Student's t-test was used to test whether BMD was linearly related to milk consumption (ordinal values). Visual inspection of model residual suggested that use of z-scores was justifiable.

Data are presented unadjusted and adjusted for age, midlife or current BMI, past and present physical activity, alcohol consumption and cod liver oil consumption.

For stability analyses individuals taking medication known to affect bone health at the time of AGES examinations, 435 men (21%) and 992 women (37%), were excluded. The list of medications that resulted in exclusion for this secondary analysis was antiepileptic medication, calcium supplements, oral estrogens, glucocorticoids, osteoporosis drugs, prostate disease drugs, proton pump inhibitors, oral steroids and thyroid agonists). Statistical analyses were conducted in SAS (version 9.2; SAS Institute Inc., Cary, NC, USA).

Results

Possible confounders, in relation to category of milk consumption in adolescence, midlife and current old age, are shown in Table 1. Consumption of milk was common at all periods of life, though the proportion of participants reporting any intake decreased with age. Daily consumption decreased from 77% in adolescence to 59% in midlife and 49% in current old age. Only 1.5% of our participants reported no intake in adolescence, 3.5% in midlife and 14% reported no current consumption.

The relationship between different levels of milk consumption and Z-scores calculated from hip BMD in old age, is shown in Table 2. Data is shown separately for men and women and both unadjusted and adjusted for confounders. Individuals with the highest frequency of milk consumption in adolescence (≥once/day) had higher Z-scores by 0.08-0.15 on average compared to those in the lowest intake group (<once/week). The difference was insignificant for both femoral neck and trochanter, and both genders. Men with the highest frequency of milk consumption during midlife had significantly higher Z-scores by 0.21 and 0.22 for femoral neck and trochanter respectively, compared to those with the lowest frequency of consumption. Comparable differences in Z-scores for women were 0.20 and 0.18 for femoral neck and trochanter respectively. For femoral neck for women, those with midlife consumption of 1-6 times/week also had significantly higher Z-scores compared to women in the lowest intake group. For current consumption, individuals with the highest frequency of consumption had Z-scores approximately 0.09 higher than those in the lowest intake group, the differences were similar for femoral neck and trochanter.

In the adjusted data, there was a dose response between milk consumption at each period of time and Z-scores calculated from hip BMD in old age. When comparing individuals constantly with the highest versus the lowest frequency of intake in the three different periods of life included in the study, the difference in BMD for both femoral neck and trochanter was similar to the difference seen for midlife consumption (data not shown).

Frequency of milk consumption at any period of time assessed here was not found to be significantly associated to femoral neck area (Additional data).

Almost one third of the participants were taking medications known to be able to affect bone health. Proton pump inhibitors were most common (13%), followed by thyroid agonists, osteoporosis related drugs, oral estrogens for women, and prostate disease drugs for men. We therefore also performed analysis without these individuals reaching the same conclusions as in our primary analysis where these subjects were included.

Discussion

This study assessed the association between milk consumption at different periods of life and hip BMD in old age. Our findings suggest that regular milk consumption throughout life, from adolescence to old age, is associated with higher BMD in old age. The strongest association was seen for midlife milk consumption, and slightly stronger for men than women.

The midlife period in our study (40-50y) probably includes early menopause for most women in our study, and those years are characterized by rapid bone loss, largely due to decreased estrogen production [29-33]. While some studies have found calcium interventions to be more effective in late postmenopausal women compared to peri-menopausal [34], we found the strongest association between frequent milk consumption during midlife and BMD in old age. A comparable relationship has also been found in another cross-sectional study by Soroko et al. [14].

It has been estimated that a 1 SD decrease in hip BMD is associated with approximately 2.5 fold increased risk of hip fracture [35,36]. Thus the difference of 0.2 Z-scores (equal to 0.2 SD), seen between individuals with high versus low frequency of milk intake in midlife, may be considered as clinically relevant, possibly associated with a 20-30% increased risk in the lowest intake group, assuming linear relationship between BMD and fracture risk.

Men lose bone minerals from all skeletal sites after approximately 50 years of age, even though their bone loss is more gradual than for women [37,38]. The stronger association observed between midlife milk consumption and BMD in old age among men than women was therefore somewhat surprising. Possibly, the stronger association seen for men might be related to the validity of the AGES-FFQ, as men’s answers to questions on milk consumption were found to be of greater validity than those for women [21,22]. The association seen for men may therefore reflect an even more accurate relationship than for women.

The interest in milk consumption and its association to BMD is primarily due to the relationship between BMD and osteoporosis and fracture risk. Osteoporosis is an important health problem for men as well as women [39], while studies on bone health among the elderly have generally focused on women. Intervention studies have shown that calcium supplementation in elderly women can have a small benefit on age-related bone loss [40,41], and milk supplementation may diminish bone turnover among postmenopausal women [42].

As milk consumption is just as strongly related to BMD for men as women in the present study, it is plausible that calcium supplementation and/or increased milk consumption among men would have a comparable effect as among women.

Similar to findings of other cross-sectional and cohort studies on milk intake in childhood and/or adolescence and bone health in adulthood and later life for women [11-16], we also found a positive association between milk consumption in adolescence and BMD in old age, though insignificant. One should however bear in mind that retrospective assessment of milk intake with more than 60 years of temporal separation is always going to provide less precise estimates and is likely to mask or weaken any potential association.

Consumption of milk and dairy products is a good indicator of calcium intake in Iceland, both during present and earlier times. Traditionally, milk consumption has been high, while other calcium sources have been limited, including a calcium-depleted water supply (4.8mg/L), and minimal consumption of green vegetable or calcium rich small fish [28,43,44]. Furthermore, calcium fortification has not been common and was virtually non-existent during the participants' adolescence and midlife, and according to INDSs approximately 70% of dietary calcium has been obtained from milk and dairy products, including cheese. The consumption of cheese was relatively low in earlier times, but has been rather stable over the past two decades, with average consumption of approximately 35-40g/d [28,43,44]. It can therefore be assumed that those participants consuming little or no milk or dairy products most likely had low calcium intakes.

It should be mentioned that milk is generally not fortified with vitamin D in Iceland, except for one low fat milk product that came on the market in the 1980s, containing 0.36µg/100ml and another one introduced in 2012 containing 1µg/100ml. The milk consumed in adolescence or midlife by our study participants therefore did not contain vitamin D of any consequence. Dietary sources of vitamin D are few, and according to the 2010-2011 Icelandic National Dietary Survey (INDS) [44] average intake of vitamin D among 61-80 year-old individuals, not counting supplements, was 5.3µg/day. Due to the lay of the land dermal production of vitamin D is also limited or non-existent from approximately October to April [45]. There is a tradition of using cod liver oil as a vitamin D supplement in Iceland, and we adjusted for intake in our analysis. However, leaving it out of adjustments only resulted in minor changes in the association seen between milk intake and hip BMD.

The AGES-Reykjavik study, with its large number of participants and relatively high proportion of men, provided a unique opportunity to assess the association between milk consumption of different periods of life and bone health in old age of both sexes. Also, extensive data gathered in the AGES-Study, and midlife data received from the Reykjavik-Study, allowed for adjustments of various confounding factors.

The retrospective assessment of milk consumption is the main limitation of the study. The AGES-FFQ on midlife and current intake has been validated and milk and dairy products were among the food items with the highest correlation to the reference methods in both instances [21,22], but the part of the AGES-FFQ asking about adolescent intake (14-19y) has not and will not be assessed for validity, as it is extremely difficult to do. However, studies have shown that there is not necessarily a clear decline in accuracy of reports with increased time lag, and recalled diet from childhood, with 50 years of temporal separation, may be fairly accurate [46]. Furthermore, data from the AGES-FFQ on milk intake has been used in cancer risk studies, where frequent intake during adolescence was associated with a three-fold increased risk of advanced prostate cancer in old age, while no significant risk differences were associated with milk intake during later periods of life [47]. This further supports our conclusion that milk intake in adolescence is reported with sufficient accuracy to rank individuals according to level of consumption and identify high and low consumers for studying diet-related disease risk.

The inability to accurately assess amount consumed, is another limitation of the study, since the AGES-FFQ includes only questions on frequency of intake. As a result we are only able to rank individuals into higher and lower intake groups, without defining the specific amounts of milk or dairy. Still, the comparisons being made between the lowest milk intake (<once/week) and the highest (≥once/day) can be of practical relevance and ranking individuals according to intake is a common practice in epidemiologic research [47].

Milk and dairy products are nutrient dense foods, also supplying other nutrients and factors besides calcium, that may promote bone growth and calcium accretion, such as proteins, peptides, phosphates, potassium and magnesium, as well as growth factors and other hormones [48,49]. Possibly milk also contains other components yet to be identified that affect bone density. Thus the observed association between milk consumption and BMD may possibly be explained by other factors besides calcium intake. While the significance of milk intake at various ages for bone health in old age warrants further study, we believe that the differences seen here in BMD between elderly people according to their milk intake throughout life, and especially during midlife, may have practical relevance for public health.

Conclusion

Our data suggest that milk consumption throughout life, and especially during midlife, has long-term association with hip BMD in old age. As BMD has been shown to be a predictor of fracture risk, we consider the difference seen in BMD between individuals with more versus less frequent milk consumption to be of clinical relevance for both men and women.

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Table 1. Possible confounding factors in relation to milk consumption in adolescence (14-19y), midlife (40-50y) and current consumption. Data shown as mean (SD) / median (IQR)*, or proportion (%).

	Men			Women		
	<once/week	1-6/week	≥once/day	<once/week	1-6/week	≥once/day
Adolescence						
Age, y	77.5 (6.2)	76.5 (5.2)	76.4 (5.4)	76.4 (6.2)	75.7 (5.3)	76.2 (5.6)
Midlife BMI, kg/m^2	26.1 (3.0)	25.6 (3.0)	25.5 (3.2)	24.8 (6.1)*	24.4 (4.7)*	24.2 (4.4)*
Current BMI, kg/m^2	26.8 (3.9)	26.8 (3.7)	26.8 (3.8)	28.2 (6.9)*	27.1 (6.0)*	26.7 (5.9)*
Alcohol, g/week	3.2 (30.8)*	6.4 (26.4)*	6.4 (26.4)*	1.6 (6.4)*	1.6 (9.7)*	1.6 (8.0)*
PA present, h/w	0.03 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.0 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
PA 50-65y, h/w	0.0 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
PA 35-49y, h/w	0.0 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
PA 20-34y, h/w	0.05 (0.5)*	0.05 (1.5)*	0.05 (5.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
Daily intake of cod liver oil	30.0%	21.7%	32.9%	20.0%	23.8%	38.8%
Medications	22.5%	21.8%	20.4%	43.2%	36.7%	36.4%
Midlife						
Age, y	76.6 (5.6)	76.1 (5.2)	76.6 (5.4)	76.5 (5.8)	75.6 (5.2)	76.4 (5.6)
Midlife BMI, kg/m^2	25.8 (3.2)	25.6 (3.2)	25.4 (3.1)	24.5 (4.9)*	24.3 (4.4)*	24.2 (4.5)*
Current BMI, kg/m^2	27.2 (4.1)	27.0 (3.7)	26.6 (3.8)	27.5 (6.3)*	27.0 (5.9)*	26.6 (5.9)*
Alcohol, g/week	6.4 (33.0)*	8.0 (24.8)*	4.8 (26.4)*	1.6 (16.1)*	1.6 (9.7)*	1.6 (8.0)*
PA present, h/w	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.0 (0.05)*	0.05 (1.5)*	0.0 (1.5)*
PA 50-65y, h/w	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
PA 35-49y, h/w	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
PA 20-34y, h/w	0.05 (1.5)*	0.05 (5.5)*	0.05 (5.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
Daily intake of cod liver oil	42.1%	34.4%	48.9%	37.8%	39.6%	46.2%
Medications	23.6%	19.6%	20.9%	35.3%	36.9%	37.0%
Current						
Age, y	76.3 (5.4)	75.9 (5.1)	76.8 (5.4)	76.0 (5.6)	75.9 (5.3)	76.3 (5.6)
Midlife BMI, kg/m^2	25.4 (3.4)	25.6 (3.0)	25.5 (3.1)	24.2 (4.4)*	24.3 (4.4)*	24.4 (4.5)*
Current BMI, kg/m^2	26.5 (3.9)	27.0 (3.6)	26.8 (3.8)	26.6 (6.0)*	26.8 (6.1)*	26.9 (5.8)*
Alcohol, g/week	8.0 (32.8)*	8.0 (24.8)*	4.8 (24.1)*	3.2 (13.2)*	1.6 (8.0)*	1.6 (6.4)*
PA present, h/w	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.0 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
PA 50-65y, h/w	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
PA 35-49y, h/w	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
PA 20-34y, h/w	0.05 (5.5)*	0.05 (4.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*

Daily intake of cod liver oil	59.8%	55.6%	63.6%	54.2%	57.5%	65.7%
Medications	19.8%	17.0%	22.7%	35.9%	36.1%	37.7%

Medications: Reported intake of medication known to affect bone density

Table 2. Difference in Z-scores, derived from hip BMD, between individual with milk consumption of 1-6 times/week and ≥once/day compared to <once/week.

	Femoral neck				Trochanteric area			
	Men		Women		Men		Women	
	Δ Z	95% CI	Δ Z	95% CI	Δ Z	95% CI	Δ Z	95% CI
Unadjusted								
Adolescence								
<once/week	referent	-	-	-	-	-	-	-
1-6 week	0.11	-0.21 ; 0.44	0.10	-0.11 ; 0.30	0.02	-0.30 ; 0.34	0.10	-0.11 ; 0.30
≥once/day	0.15	-0.16 ; 0.46	0.08	-0.12 ; 0.27	0.09	-0.22 ; 0.40	0.09	-0.10 ; 0.28
p for trend		0.29		0.85		0.19		0.68
Midlife								
<once/week	referent	-	-	-	-	-	-	-
1-6 week	0.17	-0.02 ; 0.35	0.17	0.02 ; 0.31	0.16	-0.03 ; 0.34	0.17	0.03 ; 0.31
≥once/day	0.20	0.02 ; 0.37	0.17	0.03 ; 0.30	0.20	0.02 ; 0.37	0.15	0.01 ; 0.28
p for trend		0.05		0.09		0.04		0.23
Current								
<once/week	referent	-	-	-	-	-	-	-
1-6 week	-0.01	-0.13 ; 0.12	0.06	-0.05 ; 0.16	0.05	-0.07 ; 0.17	0.09	-0.02 ; 0.19
≥once/day	0.08	-0.02 ; 0.19	0.06	-0.03 ; 0.15	0.09	-0.02 ; 0.19	0.08	-0.01 ; 0.17
p for trend		0.08		0.23		0.11		0.11
Adjusted*								
Adolescence								
<once/week	referent	-	-	-	-	-	-	-
1-6 week	0.11	-0.21 ; 0.43	0.11	-0.08 ; 0.30	0.004	-0.31 ; 0.32	0.11	-0.08 ; 0.29
≥once/day	0.15	-0.16 ; 0.45	0.12	-0.06 ; 0.30	0.08	-0.22 ; 0.38	0.13	-0.04 ; 0.31
p for trend		0.29		0.31		0.17		0.17
Midlife								
<once/week	referent	-	-	-	-	-	-	-
1-6 week	0.15	-0.02 ; 0.33	0.14	0.002 ; 0.27	0.15	-0.03 ; 0.32	0.13	-0.004 ; 0.26
≥once/day	0.21	0.04 ; 0.38	0.20	0.07 ; 0.33	0.22	0.05 ; 0.38	0.18	0.05 ; 0.30
p for trend		0.02		0.002		0.008		0.007
Current								

<once/week	referent	-	-	-	-	-	-	-
1-6 week	-0.02	-0.14 ; 0.10	0.06	-0.03 ; 0.16	0.03	-0.09 ; 0.15	0.09	-0.002 ; 0.18
≥once/day	0.09	-0.01 ; 0.20	0.07	-0.01 ; 0.16	0.09	-0.01 ; 0.20	0.09	0.01 ; 0.17
p for trend		0.04		0.12		0.05		0.04

*Data adjusted for age, past and present physical activity, alcohol consumption, cod liver oil intake, midlife BMI for adolescence and midlife consumption and current BMI for current consumption,

Additional file. Femoral neck area, difference in Z-scores, derived from BMD, between individuals with milk consumption of 1-6 times/week and ≥once/day compared to <once/week.

	Femoral neck area			
	Men		Women	
	Δ Z	95% CI	Δ Z	95% CI
Unadjusted				
Adolescence				
<once/week	referent	-	-	-
1-6 week	-0.12	-0.44 ; 0.21	-0.03	-0.23 ; 0.18
≥once/day	-0.06	-0.38 ; 0.25	0.04	-0.15 ; 0.23
Midlife				
<once/week	referent	-	-	-
1-6 week	-0.05	-0.23 ; 0.13	0.01	-0.13 ; 0.16
≥once/day	-0.02	-0.20 ; 0.16	-0.02	-0.16 ; 0.12
Current				
<once/week	referent	-	-	-
1-6 week	-0.03	-0.15 ; 0.10	0.07	-0.04 ; 0.17
≥once/day	-0.01	-0.12 ; 0.09	-0.01	-0.10 ; 0.08
Adjusted*				
Adolescence				
<once/week	referent	-	-	-
1-6 week	-0.12	-0.45 ; 0.20	-0.02	-0.23 ; 0.18
≥once/day	-0.05	-0.37 ; 0.26	0.06	-0.13 ; 0.26
Midlife				
<once/week	referent	-	-	-
1-6 week	-0.04	-0.22 ; 0.15	0.01	-0.13 ; 0.16
≥once/day	-0.01	-0.18 ; 0.17	0.001	-0.14 ; 0.14
Current				
<once/week	referent	-	-	-
1-6 week	-0.03	-0.16 ; 0.10	0.06	-0.04 ; 0.17
≥once/day	-0.02	-0.13 ; 0.08	0.003	-0.09 ;0.10

Additional file. QCT measurements of left hip

	Men			Women		
	P10	**P50**	**P90**	**P10**	**P50**	**P90**
Femoral neck integral BMD (g/cc)	0.19	0.25	0.32	0.18	0.24	0.31
Femoral neck area (cm2)	7.05	8.80	11.70	5.62	7.10	9.16
Trochanter integral BMD (g/cc)	0.19	0.25	0.32	0.17	0.22	0.29

IV

Title page

Lifetime consumption of cod liver oil and bone mineral density in old age.

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Abstract

Background: Cod liver oil is a traditional source of vitamin D in Iceland, and intake is recommended partly for the sake of bone health. However, the association between lifelong consumption of cod liver oil and bone mineral density (BMD) in old age is unclear. In this study the association between intake of cod liver oil at different periods of life (adolescence, midlife, and current old age) and hip BMD in old age, as well as the association between current cod liver oil intake and serum 25(OH)D concentration was assessed.

Methods: Participants of the AGES-Reykjavik Study, age 66-96 years (N=4798), reported retrospectively cod liver oil intake during adolescence and midlife, as well as intake in current old age, using a validated food frequency questionnaire. BMD of femoral neck and trochanteric area was measured by volumetric quantitative computed tomography (QCT) and serum 25(OH)D concentration was measured by means of a direct, competitive chemiluminescence immunoassay. Associations were assessed using linear regression models.

Results: No significant association was seen between retrospective cod liver oil intake and hip BMD in old age. Current intake for men was not associated with hip BMD, while women with daily intakes had Z-scores on average 0.1 higher compared with those with intake of <once/week. Current intake was positively associated with serum 25(OH)D, individuals with intake of <once/week, 1-6 times/week and daily intake having concentrations of approximately 40nmol/L, 50nmol/L, and 60nmol/L respectively (P for trend <0.001).

Conclusion: Retrospective cod liver oil intake in adolescence and midlife was not associated with hip BMD in old age. Current intake was significantly associated with BMD in women, but not in men. Current intake was also positively associated with serum 25(OH)D concentration.

Key words: cod liver oil, lifelong consumption, bone density, elderly

Introduction

Vitamin D is important for bone health through the actions of its hormonal form, 1,25$(OH)_2$D, elevating serum calcium and phosphorus levels necessary for bone mineralization.

Cod liver oil is a traditional source of vitamin D in many Nordic countries, including Iceland. As the country lies at 63-67°N, little or no vitamin D is synthesized in the skin from approximately October to April [1]. Dietary sources are also limited, and milk and dairy products are generally not fortified with vitamin D. Supplements are therefore especially important, and the intake of cod liver oil, or other vitamin D containing supplements, is recommended for people of all ages [2].

Whether, and then to what extent, cod liver oil intake at different periods of life is related to variations in bone mineral density (BMD) in old age is not clear as there are few studies focusing on this relationship. Findings from a Norwegian follow-up study suggested that childhood cod liver oil intake may be associated with adverse effects on BMD in elderly women, supposedly due to the high vitamin A (in the form of retinol) content of cod liver oil in earlier times [3]. However, as studies are few, it is still not clear whether cod liver oil intake during childhood, supplying high amounts of both retinol and vitamin D, has adverse effects on bone health.

Studies are also few on the association between cod liver oil intake in adulthood or old age and bone density. Existing studies on intake of elderly women have found no adverse effects on BMD [4,5] and one study even found an association with lower overall fracture risk [6]. However, these studies did not involve intake in childhood or adolescence, and it is possible that the growing bone may be differently affected than adult bone. Furthermore, studies on cod liver oil intake and BMD of elderly men are completely lacking.

It is of public health importance to assess possible effects of lifelong cod liver oil intake on bone, particularly as bone health is the primary justification for recommending and taking cod liver oil. The aim of this study was to assess whether retrospective self-reports of cod liver oil intake during adolescence and midlife, are associated with hip BMD in old age. Further, the association between intake of cod liver oil in current old age and hip BMD, among participants of the Age, Gene/Environment Susceptibility – Reykjavik (AGES-Reykjavik) Study was investigated. The AGES-Reykjavik Study is a large epidemiological study, including 5,764 elderly participants with extensive health related data for all participants, including that on bone health and serum 25(OH)D concentrations, as well as data on dietary intake in adolescence, midlife and old age.

Methods

Subjects - Design

The AGES–Reykjavik Study originates from the Reykjavik Study, a large population-based cohort study which was launched in 1967. All men and women born in 1907-1935 (n=30,795) and residing in Reykjavik and nearby communities in 1967 were selected, 27,281 were invited to participate and 19,381 attended [7-10]. Of the 11,549 previously examined Reykjavik Study cohort members still alive when AGES–Reykjavik examinations began in 2002, 8,030 individuals were randomly chosen and invited to participate. When AGES– Reykjavik examinations concluded in 2006, 5,764 individuals (72%) had been enrolled and examined (42% male). Participants were 66-96 years old at time of examinations, average age being 76 years.

The AGES–Reykjavik examination was completed in three clinic visits within a 4- to 6-week time window. Extensive data were collected during clinical examinations, e.g. on physical and cognitive function, anthropometry, health history, and food history during adolescence, midlife and in current old age. Participants also underwent quantitative computerized tomography (QCT-scans) and were asked to bring to the clinic all medications and supplements used in the previous two weeks, representing current usage [8,11].

Of the 5,764 participants, 933 individuals did not undergo the QCT scanning, and additional 33 individuals did not give adequate dietary information. Therefore data from 4,798 individuals (44% male) was used in the present study.

The AGES-Reykjavik Study was approved by the Icelandic National Bioethics Committee (VSN: 00-063) and the MedStar IRB for the Intramural Research Program, Baltimore, MD.

Measurements of serum 25-hydroxyvitamin D - 25(OH)D

Blood samples were drawn at recruitment into the AGES Study, i.e., in current old age. Measurement of 25(OH)D was conducted by means of a direct, competitive chemiluminescence immunoassay using the DiaSorin LIAISON 25(OH)D TOTAL assay (DiaSorin, Inc., Stillwater, Minnesota).

Bone mineral density/ Bone variables

Quantitative computed tomography (QCT) measurements, providing true volumetric density, were performed on the left hip using a 4-detector CT system (Sensation, Siemens Medical

Systems, Erlangen Germany). Scans were acquired using a standardized protocol and encompassed the proximal femur from a level 1cm above the acetabulum to a level 5mm inferior to the lesser trochanter with 1mm slice thickness. Further procedures and quality assessments have been described in detail elsewhere [8,12].

The variables used in the present study are volumetric integral BMD (g/cc), reflecting both trabecular and cortical bone mass, of femoral neck and trochanteric area, encompassing both trochanters. Reasons for exclusion from the QCT were inability to lie supine or weight over 150kg. Furthermore, hip scans were not performed on individuals that had undergone hip replacement surgery.

Dietary information

Dietary data were gathered using a short food frequency questionnaire (AGES-FFQ) designed for the AGES-Reykjavik Study. The questionnaire is divided into three parts, including 16 questions on adolescent intake (14-19y), 17 questions on midlife intake (40-50y) and 30 questions on current intake. Foods and food groups were selected for the questionnaire on the basis of their contribution to the absolute intake of elderly Icelanders according to former National Nutrition Surveys [13], as well as unique nutritional qualities and possible connection to the development of various diseases in later life. The questionnaire has been described previously [14,15]. Frequency of cod liver oil intake was measured by posing a question in each part of the AGES-FFQ. The response categories were: 1) Never, 2) Less than once a week, 3) 1-2 times a week, 4) 3-4 times a week, 5) 5-6 times a week or 6) Daily. (Figure 1).

Validity of the parts of the AGES-FFQ relating to midlife and present intake has been assessed in previous papers [14,15]. Cod liver oil was among the items showing the highest validity in the questionnaire. When assessing validity of questions on midlife diet, frequency of intake reported in the AGES-FFQ by 56-72-year old individuals was compared with detailed dietary data, gathered from the same individuals 18-19 years previously, i.e. in midlife, as a part of a national dietary survey [13]. Correlation using Spearman's rho was $r=0.53$, $p=<0.001$; $r=0.56$, $p=<0.001$ for men and women respectively [14]. Validity of questions on current intake was assessed among elderly individuals (average age 74y) by comparing answers of the AGES-FFQ to 3-day weighed food records completed by the same individuals. Correlation using Spearman's rho was $r=0.51$, $p<0.001$; $r=0.42$, $p <0.001$ for men and women respectively [15].

Covariates

For examining the association between intake of cod liver oil through different periods of life and hip BMD we selected *a priori* the following set of covariates: physical activity during different periods of life, current alcohol intake, age, body mass index (BMI), and milk consumption at the same period of life. Midlife BMI was chosen as a covariate for the retrospective data, and current BMI for current data.

To obtain history of physical activity, participants were asked how much time (hours/week) they spent on moderate to vigorous activities in four different periods of life; young adulthood (20-34y), early middle age (35-49y), late middle age (50-64y) and current physical activity. Both weight bearing and non-weight bearing exercises were included. Current consumption of alcohol was converted into grams per week using 14g of alcohol as a standard drink and was divided into <25g/week, 25-50g/week, and >50g/week. Midlife data on BMI had been collected in the Reykjavik-Study [7].

For early life most of these covariates can only be considered surrogate measures of corresponding early life characteristics. On the other hand for midlife and in current old age covariates selected are potential predictors of both bone health and dietary habits.

Data analysis – Statistical analysis

Characteristics of study participants were described using mean and standard deviation (SD) of normal variables, median and interquartile range (IQR) for skewed variables and percentages for dichotomous variables.

Due to the approximate normal distribution of the source BMD variables in our population they were transformed into sex-specific z-scores, reflecting the number of standard deviations (SD) from the mean BMD in our population of 66-96 years of age. Univariate and multivariate linear regression was then used for examining the association between intake of cod liver oil and BMD variables.

Intake of cod liver oil according to the AGES-FFQ was categorized into three groups; never or <once/week, 1-6 times a week, and daily intake. The lowest intake group (never or <once/week) was in all cases used as referent and results represented as difference in z-scores (Δ) with higher frequency of consumption compared to the referent. Student's t-test was used to test whether BMD was linearly related to cod liver oil intake (ordinal values). Visual inspection of model residual suggested that use of z-scores was justifiable.

Data are presented unadjusted and adjusted for age, midlife or current BMI, past and present physical activity, alcohol consumption, milk consumption at the same period of life.

For stability analyses individuals taking medication known to affect bone health at the time of AGES examinations, 435 men (21%) and 992 women (37%), were excluded. The list of medications that resulted in exclusion for this secondary analysis was antiepileptic medication, calcium supplements, oral estrogens, glucocorticoids, osteoporosis drugs, prostate disease drugs, proton pump inhibitors, oral steroids and thyroid agonists). Statistical analyses were conducted in SAS (version 9.2; SAS Institute Inc., Cary, NC, USA).

Results

Potential confounding factors in relation to cod liver oil intake at different periods of life are shown in Table 1. Intake of cod liver oil was fairly common, with the proportion of participants reporting any intake increasing with age, as 61%-70%-74% reported intake in adolescence, midlife, and current old age respectively, and 33%-43%-60% reporting daily intake.

The association between retrospective intake of cod liver oil and difference in Z-scores, calculated from hip BMD in old age (using <once/week as a referent) is shown in Table 2. Data are shown separately for men and women and both unadjusted and adjusted for confounders. Individuals taking cod liver oil more frequently in adolescence and/or midlife did not have significantly different hip BMD in old age compared with those with the lowest frequency of intake. This was seen for both men and women, and for femoral neck and trochanter.

The association between cod liver oil intake in current old age and hip BMD was also assessed (Table 3), excluding supplement users from the analysis. There was no significant difference in hip BMD in relation to cod liver oil intake for men, while women with daily intake had significantly higher Z-scores on average (0.11 for femoral neck and 0.10 for trocanther) compared to those with the lowest frequency of intake (<once/week).

There was a clear association between current intake of cod liver oil and serum 25(OH)D concentrations (Table 4). Median concentrations for those with the lowest frequency of intake being 40.2 and 37.8nmol/L for men and women respectively, compared to 61.9 and 56.4nmol/L for those with daily intake. When excluding individuals taking multisupplements from the analysis, median serum 25(OH)D concentrations for those with the lowest cod liver oil intake were 37.2 and 31.9nmol/L for men and women respectively, compared with 60.6 and 55.2nmol/L for those with high intake of cod liver oil. Both men and women with intake of 1-6 times/week or daily intake had significantly higher serum levels than those with the lowest frequency of intake.

Almost one third of the participants were taking medications known to be able to affect bone health. Proton pump inhibitors were most common (13%), followed by thyroid agonists, osteoporosis related drugs, oral estrogens for women, and prostate disease drugs for men. We therefore also performed analysis without these individuals reaching the same conclusions as in our primary analysis where these subjects were included.

Discussion

No significant association was found in the present study, between retrospective intake of cod liver oil in adolescence or midlife and hip BMD among the elderly participants of the AGES-Reykjavik Study. Current intake was also not associated with hip BMD in men, while women with daily intake had significantly higher Z-scores of both femoral neck and trochanter compared to those with the lowest frequency of consumption. Current cod liver oil intake of both sexes was positively associated with serum 25(OH)D concentration.

Adequate vitamin D is important in adolescence for bone accretion associated with rapid growth [16]. Still, intervention studies are limited, with some studies showing that vitamin D supplementation can increase bone mineral content and BMD of young adolescent girls [17,18], while other studies show no significant effect [19].

In the early and mid 20th century, when our participants were in adolescence, cod liver oil was an important dietary source of vitamin D, possibly more so than nowadays, as both food fortification and supplement use was limited. However, cod liver oil is not only rich in vitamin D and long chain omega-3 fatty acids, but is also a generous source of vitamin A, in the form of retinol. Although epidemiological studies are inconsistent, high intakes of retinol have repeatedly been linked to adverse effects on bone health and even increased risk of osteoporosis and osteoporotic fractures [20-24].

Importantly, the vitamin content of cod liver oil was quite different in the mid 20th century, compared with its present composition, as there was limited standardization of the product, and the concentrations of retinol and vitamin D probably reflected that of pure cod liver, 83:1 [25]. In later years and up until 2002, cod liver oil in Iceland still contained high amounts of retinol, or approximately 30.000µg and 250µg/100g of vitamin D. However, this high amount of retinol has since been decreased considerably and today's concentrations are 5.000 µg of retinol and 200µg of vitamin D/100g. Hence, prior to 2002 the recommended spoonful per day of cod liver oil contained approximately 1650µg of retinol, exceeding the recommended daily intake for adolescents and adults of 900µg for men and 700µg for women. According to the 1990 INDS average intake of vitamin A was approximately three times the recommended daily intake [13], but has since decreased, both as a result of the decreased concentration of retinol in cod liver oil, as well as decreased intake of whole milk, margarine, and other vitamin A rich foods [26,27].

Cod liver oil is a traditional source of vitamin D in other Nordic countries as well as Iceland. In a follow-up study of 50-70 year old women in the Norwegian Nord-Trøndelag Health Study, an association was found between childhood consumption of cod liver oil and current forearm BMD, where elderly women reporting any intake in childhood had significantly lower BMD than those with no intake [3]. The researchers concluded that the previously high concentration of vitamin A in cod liver oil, when added to an already vitamin A rich diet, may have lead to total intake reaching harmful levels.

In the light of the results from the Nord-Trøndelag Health Study, we set out to explore whether those findings could be replicated using Iceland data, since comparable conditions have existed here with respect of vitamin A intake and use of cod liver oil. In short, we did not find any indication that the intake of cod liver oil during adolescence or midlife was associated with adverse effects on BMD of either femoral neck or trochanter. Further, we did not find any association between current intake and BMD in old age for men, while there was a positive association found for women.

It should be noted that different methods were used for measuring BMD in the two studies. Also, the Nord-Trøndelag Health Study measured forearm BMD, while we use hip BMD, and it is possible that these bones respond differently to cod liver oil intake. Hip bones have for an example been reported to show greater response to external factors, e.g. vitamin D, than other skeletal sites [28].

Intervention studies on vitamin D supplementation among elderly individuals have shown that increased intake can be associated with increased BMD [29,30], decrease bone loss [30-32], and lower risk of osteoporotic fractures [30-34]. However, many of the intervention studies showing increases in BMD associated with vitamin D also include calcium supplements parallel to the vitamin D, making interpretations difficult. Previous studies of elderly women have not shown any association between current cod liver oil intake and BMD [5,35]. While our results showed no association between current cod liver oil intake and hip BMD for men, there was a slight positive association for women. It has been estimated that a 1 SD decrease in hip BMD is associated with approximately 2.5 fold increased risk of hip fracture [36,37]. Thus the difference of 0.1 Z-scores (equal to 0.1 SD), seen between women with daily intake versus <once/week may be of clinical relevance.

The rather weak association found between current cod liver oil intake and hip BMD in our study may possibly be explained by the relatively high serum 25(OH)D levels in our population. Even for individuals with no intake of cod liver oil less then <once/week, median

serum levels were approximately 40nmol/L, which is close to the 50nmol/L considered adequate for bone health by the Institute of Medicine [16]. Furthermore, in previous Icelandic studies, serum parathyroid hormone (PTH) levels off at approximately 45-50nmol/L in healthy adult and elderly individuals, suggesting vitamin D sufficiency with respect to bone health [38,39].

The median serum concentration observed in the present study (52nmol/L) is also in line with what has previously been reported for elderly Icelanders [39]. Thus the relatively adequate vitamin D status of our older population, even in those taking neither cod liver oil nor other supplements, may possibly mask any putative benefit of cod liver oil intake.

The association between current frequency of intake and serum 25(OH)D concentrations may be considered as further validation of the question on cod liver oil in the AGES-FFQ, as concentrations increased with increased frequency of intake of cod liver oil. Participants with intake of <once a week, 1-6 times a week and daily intake having serum levels of approximately 40nmol/L, 50nmol/L and 60nmol/L respectively. The validity of questions of the AGES-FFQ on current and midlife intakes has been assessed previously, and in both instances cod liver oil was among those items that had the highest correlation with the referent methods [14,15]. Adolescent intake however has not, and will not be validated due to methodological difficulties.

The AGES-Reykjavik study, with its large number of participants and relatively high proportion of men, provided a unique opportunity to assess the association between cod liver oil intake in different periods of life and bone health in old age of both sexes. Also, extensive data gathered in the AGES-Study, and midlife data received from the Reykjavik-Study, allowed for adjustments of various confounding factors.

The main limitation of the study is that we are partly using retrospective data with 60 years of temporal separation on average, which is always going to be imprecise and is likely to mask any potential modest or weak association. However, there is not necessarily a clear decline in accuracy of reports with increased time lag, and recalled diet from childhood, with 50 years of temporal separation, may be fairly accurate [40]. Furthermore, according to Dwyer & Coleman [40] foods, meals or supplements with special characteristics (such as cod liver oil), can be recalled particularly well. Another limitation is that we do not have absolute amount consumed of cod liver oil, as the questionnaire asks only of frequency. However, the most common intake is a spoonful per day and the variance is presumably less than for other foods e.g., meat, fish, fruit, vegetables etc when asked only of frequency and not serving sizes.

We did not have any information on supplement use during midlife, and could therefore not analyse the data separately without supplement users. Hence, we do not know is such an analysis might have yielded different results.

As we are assessing cross-sectional association between current cod liver oil intake and serum 25(OH)D concentrations, we remain cautious in our interpretation as influence of other unmeasured confounders, not included in our analysis, cannot be excluded. Outdoor activities and amount of sunlight received are examples of such factors. However, the seasonal variation observed in this study was small, or 3.9nmol, similar for men and women, and somewhat lower than seen in previous studies of adults and elderly Icelanders [1,38,39], suggesting that sun exposure is a relatively small confounding factor here.

Also, we do not have accurate enough information on total food intake in order to calculate total vitamin D intake of our participants. The AGES-FFQ only includes simple global questions, such as on frequency of fish intake, without asking of different types of fish, e.g., fatty fish and lean fish. The questionnaire also does not include special questions on fortified food items. In a recent INDS the portion of the oldest age group (61-80y), not taking cod liver oil, had an average intake of 5.3µg/d of vitamin D, vitamin D from supplements not included (27). Assuming that the intake of our participants was comparable, it would suggest that 5 to 6µg/d might be sufficient to keep average serum levels of approximately 40nmol/L. This is however lower than has previously been reported where approximately 9µg/d of vitamin D have been required to achieve average serum 25(OH)D concentrations of 50nmol/L [41].

In conclusion, we found no evidence for cod liver oil at any age having a harmful effect on hip BMD in old age, while there was a small positive association with current intake and BMD in older women. Still, the significance of cod liver oil intake at various ages for bone health in old age warrants further study, especially intake during childhood and adolescence, as cod liver oil is supplied in several schools and child care centers in Iceland for public health purposes.

Conclusion

Intake of cod liver oil, containing high amounts of both retinol and vitamin D, in early and midlife does not appear to be associated with hip BMD in old age. For current intake, there was an increase of approximately 20nmol/L in 25(OH)D concentration following daily intake of cod liver oil, compared to no intake or less than once a week. Current intake of cod liver oil, containing only a sixth of the amount of retinol in earlier times, was positively associated with hip BMD in old age for women, but not for men. Possibly, the relatively high median serum 25(OH)D concentration in our study population, even among those not taking supplements or cod liver oil, may mask any putative, more profound relationship between current intake and hip BMD, especially in men who had higher serum 25(OH)D levels than women.

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Figure 1. Example of a question of cod liver oil intake in the AGES-FFQ.

On average, how often do you take cod liver oil or liver oil pills?

☐ Never

☐ Less than once a week

☐ 1-2 times a week

☐ 3-4 times a week

☐ 5-6 times a week

☐ Daily

Table 1. Possible confounding factors in relation to cod liver oil intake in adolescence (14-19y), midlife (40-50y) and current old age. Data shown as mean (SD) / median (IQR)*, or proportion (%).

	Men			Women		
	<once/week	1-6 times/week	Daily	<once/week	1-6 times/week	Daily
Adolescence						
Age, y	76.6 (5.5)	76.0 (5.3)	76.6 (5.1)	76.1 (5.7)	75.6 (5.3)	76.4 (5.4)
Midlife BMI, kg/m^2	25.4 (3.0)	25.7 (3.3)	25.5 (3.1)	24.4 (4.6)*	23.7 (4.2)*	24.4 (4.4)*
Current BMI, kg/m^2	26.7 (3.8)	27.0 (4.0)	26.7 (3.7)	26.8 (6.2)*	26.4 (5.7)*	27.0 (5.7)*
Alcohol, g/week	6.4 (26.4)*	8.0 (26.4)*	4.8 (24.1)*	1.6 (8.0)*	1.6 (8.0)*	1.6 (8.0)*
PA current, h/w	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.0 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
PA 50-65y, h/w	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
PA 35-49y, h/w	0.05 (1.5)*	0.05 (1.5)*	0.05 (5.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
PA 20-34y, h/w	0.05 (1.5)*	0.05 (5.5)*	0.5 (5.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
Daily milk consumption	78.4%	73.2%	84.7%	71.0%	72.1%	83.9%
Medications**	21.4%	19.5%	20.6%	37.1%	35.6%	37.0%
Midlife						
Age, y	76.9 (5.6)	75.4 (5.3)	76.6 (5.1)	76.1 (5.6)	75.6 (5.3)	76.3 (5.5)
Midlife BMI, kg/m^2	25.7 (3.3)	25.4 (3.1)	25.4 (3.0)	24.6 (4.6)*	23.8 (4.3)*	24.2 (4.4)*
Current BMI, kg/m^2	27.1 (4.0)	27.0 (3.8)	26.4 (3.6)	27.1 (6.0)*	26.4 (6.1)*	26.6 (5.8)*
Alcohol, g/week	4.8 (26.4)*	8.0 (26.4)*	6.4 (26.4)*	1.6 (8.0)*	3.2 (8.0)*	1.6 (8.0)*
PA current, h/w	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.0 (0.5)*	0.05 (1.5)*	0.05 (1.5)*
PA 50-65y, h/w	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
PA 35-49y, h/w	0.05 (1.5)*	0.05 (1.5)*	0.05 (5.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
PA 20-34y, h/w	0.05 (1.5)*	0.05 (1.5)*	0.5 (5.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
Daily milk consumption	59.0%	52.7%	69.3%	53.7%	52.0%	60.3%
Medications**	20.7%	19.8%	21.1%	36.3%	38.3%	36.5%
Current						
Age, y	76.6 (5.6)	75.4 (5.1)	76.6 (5.3)	76.1 (5.6)	75.4 (5.2)	76.2 (5.5)
Midlife BMI, kg/m^2	25.6 (3.5)	25.3 (3.0)	25.5 (3.0)	24.8 (4.9)*	24.2 (4.3)*	24.1 (4.3)*
Current BMI, kg/m^2	27.2 (4.1)	26.9 (3.5)	26.6 (3.7)	27.3 (6.3)*	26.6 (6.6)*	26.5 (5.7)*

Alcohol, g/week	4.8 (26.4)*	13.2 (24.8)*	6.4 (26.4)*	1.6 (6.4)*	3.2 (9.7)*	1.6 (8.0)*
PA current, h/w	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.0 (0.5)	0.05 (1.5)*	0.05 (1.5)*
PA 50-65y, h/w	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
PA 35-49y, h/w	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
PA 20-34y, h/w	0.05 (1.5)*	0.05 (1.5)*	0.05 (5.5)*	0.05 (1.5)*	0.05 (1.5)*	0.05 (1.5)*
Daily milk consumption	49.1%	47.7%	54.9%	41.6%	37.6%	51.0%
Medications**	18.1%	20.7%	22.1%	39.1%	34.4%	35.9%

*median and interquartile range

**Medications: Reported intake of medication known to affect bone density

Table 2. Difference in hip BMD in old age, presented as Z-scores, between individual with retrospective cod liver oil intake of 1-6 times/week or daily intake, compared to <once/week.

	Femoral neck				Trochanteric area			
	Men		Women		Men		Women	
	Δ Z	95% CI	Δ Z	95% CI	Δ Z	95% CI	Δ Z	95% CI
Unadjusted								
Adolescence intake								
<once/week	referent	-	-	-	-	-	-	-
1-6 week	0.09	-0.01 ; 0.20	-0.09	-0.20 ; 0.01	0.08	-0.03 ; 0.19	-0.10	-0.20 ; 0.01
daily	0.02	-0.08 ; 0.12	0.04	-0.05 ; 0.12	0.01	-0.09 ; 0.11	0.05	-0.03 ; 0.14
p-value		0.61		0.48		0.79		0.28
Midlife intake								
<once/week	referent	-	-	-	-	-	-	-
1-6 week	0.02	-0.10 ; 0.14	-0.06	-0.17 ; 0.05	-0.01	-0.13 ; 0.11	-0.06	-0.17 ; 0.06
daily	0.01	-0.09 ; 0.11	0.01	-0.07 ; 0.10	-0.01	-0.11 ; 0.09	0.01	-0.07 ; 0.09
p-value		0.83		0.73		0.84		0.83
Adjusted*								
Adolescence intake								
<once/week	referent	-	-	-	-	-	-	-
1-6 week	0.06	-0.05 ; 0.16	-0.10	-0.20 ; 0.01	0.05	-0.05 ; 0.16	-0.09	-0.19 ; 0.01
daily	0.01	-0.09 ; 0.11	0.05	-0.03 ; 0.12	-0.004	-0.10 ; 0.09	0.06	-0.01 ; 0.14
p-value		0.79		0.30		0.94		0.13
Midlife intake								
<once/week	referent	-	-	-	-	-	-	-
1-6 week	-0.03	-0.15 ; 0.08	-0.08	-0.19 ; 0.02	-0.06	-0.18 ; 0.06	-0.07	-0.17 ; 0.03
daily	-0.01	-0.11 ; 0.09	0.02	-0.06 ; 0.10	-0.03	-0.13 ; 0.06	0.02	-0.05 ; 0.10
p-value		0.84		0.58		0.53		0.51

*Adjusted for age, past and present physical activity, midlife BMI, alcohol consumption, and milk consumption at the same period

Table 3. Difference in hip BMD, presented as Z-scores, in relation to current cod liver oil intake (supplement users excluded).

		Femoral neck				Trochanteric area			
		Men		Women		Men		Women	
		Δ Z	95% CI	Δ Z	95% CI	Δ Z	95% CI	Δ Z	95% CI
Unadjusted									
	<once/week	referent	-	-	-	-	-	-	-
	1-6 week	0.06	-0.11 ; 0.23	-0.02	-0.20 ; 0.15	0.11	-0.06 ; 0.28	-0.06	-0.23 ; 0.12
	daily	-0.0003	-0.11 ; 0.11	0.05	-0.05 ; 0.15	0.02	-0.09 ; 0.13	0.03	-0.08 ; 0.13
	p-value		0.93		0.34		0.86		0.56
Adjusted									
	<once/week	referent	-	-	-	-	-	-	-
	1-6 week	0.04	-0.13 ; 0.20	-0.07	-0.23 ; 0.10	0.10	-0.07 ; 0.26	-0.12	-0.28 ; 0.04
	daily	-0.02	-0.13 ; 0.09	0.11	0.01 ; 0.20	0.02	-0.09 ; 0.12	0.10	0.01 ; 0.19
	p-value		0.69		0.02		0.88		0.02

*Adjusted for age, past and present physical activity, current BMI, alcohol consumption, and current milk consumption

Table 4. Serum 25(OH)D concentration in nmol/L in relation to current cod liver oil intake

	men				women			
	n	median	P10	P90	n	median	P10	P90
All subjects								
<once/week	642	40.2	20.3	70.8	933	37.8	17.1	67.8
1-6	256	48.3	27.8	86.1	249	44.9	21.6	76.3
daily	1375	61.9	35.2	93.1	1856	56.4	29.2	84.4
p-value		<0.001				<0.001		
Without								
<once/week	518	37.2	19.6	67.7	669	31.9	15.2	63.5
1-6	203	48.9	28.1	86.6	174	44.0	21.3	70.1
daily	979	60.6	33.9	94.5	1168	55.2	27.7	85.6
p-value		<0.001				<0.001		

* Significantly different from the lowest intake group, p<0.0001

Appendix

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Matarlisti - útg. 2

Þátttakandanúmer

Dagsetning

Dag. Mán. Ár

Verkefnisnúmer

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Matarlisti

1. Hversu oft borðar þú að jafnaði heita aðalmáltíð?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

2. Hversu oft borðar þú að jafnaði kjöt eða hakkrétti í aðalmáltíð?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

3. Hversu oft borðar þú að jafnaði fisk eða fiskrétti í aðalmáltíð?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

4. Hversu oft borðar þú að jafnaði fiskiálegg, t.d. síld, sardínur, silung eða lax, á brauð eða í salat?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

5. Hversu oft borðar þú að jafnaði kartöflur?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

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6. Hversu oft borðar þú að jafnaði ferska ávexti?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni dag

7. Hversu oft borðar þú að jafnaði slátur, blóðmör eða lifrapylsu?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

8. Hversu oft borðar þú að jafnaði rúgbrauð, maltbrauð eða flatbrauð?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

9. Hversu oft borðar þú að jafnaði heilhveitibrauð eða annað gróft brauð?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

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10. Hversu oft borðar þú að jafnaði hafragraut eða múslí?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

11. Hversu oft borðar þú að jafnaði soðið eða steikt grænmeti?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

12. Hversu oft borðar þú að jafnaði grænmetissalat eða annað hrátt grænmeti?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

13. Hversu oft borðar þú að jafnaði kex, kökur eða sætabrauð?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

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14. Hversu oft borðar þú að jafnaði sælgæti?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

15. Hversu oft borðar þú að jafnaði sýrðar mjólkurvörur, skyr eða mjólkurgrauta?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

16. Hvaða tegund af sýrðum mjólkurvörum nota þú oftast/mest?

- ☐ Léttar vörur s.s. létt-ab, létt-jógurt eða létt-súrmjólk
- ☐ Venjulega ab-mjólk, súrmjólk eða jógúrt
- ☐ Þykkmjólk, abt-mjólk eða engjaþykkni
- ☐ Nota sjaldan eða aldrei sýrðar mjólkurvörur

17. Hversu oft drekkur þú að jafnaði mjólk, (nýmjólk, léttmjólk, undanrennu, fjörmjólk)?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

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18. Hvaða tegund af mjólk notar þú oftast/mest?

- ☐ Léttmjólk
- ☐ Nýmjólk
- ☐ Undanrennu
- ☐ Fjörmjólk
- ☐ Nota aldrei mjólk

19. Hversu oft drekkur þú að jafnaði hreinan ávaxtasafa?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

20. Hversu oft drekkur þú að jafnaði sykraðan djús eða gosdrykk?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

21. Hversu oft tekur þú að jafnaði þorska- eða ufsalýsi eða lýsispillur (á samt ekki við lúðulýsispillur)?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega

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22. Hversu marga bolla drekkur þú að jafnaði af kaffi **á dag**?

Ath breytta tímaviðmiðun

- ☐ Aldrei
- ☐ Minna en 1 á dag
- ☐ 1-2 á dag
- ☐ 3-4 á dag
- ☐ 5-6 á dag
- ☐ 7 eða fleiri á dag

23. Hversu marga bolla drekkur þú að jafnaði af tei **á dag**?

- ☐ Aldrei
- ☐ Minna en einn bolla á dag
- ☐ 1-2 á dag
- ☐ 3-4 á dag
- ☐ 5-6 á dag
- ☐ 7 eða fleiri á dag

24. Hversu **margar teskeiðar** notar þú af sykri í kaffi eða te á dag?

- ☐ Aldrei
- ☐ Minna en eina teskeið á dag
- ☐ 1-2 á dag
- ☐ 3-4 á dag
- ☐ 5-6 á dag
- ☐ 7 eða fleiri á dag

25. Hvaða tegund af viðbiti notar þú oftast/mest á brauð eða með mat?

- ☐ Létt og laggott
- ☐ Léttu, létt-sólblóma, klípu
- ☐ Smjör, smjörva
- ☐ Annað viðbit
- ☐ Nota ekki viðbit

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26. Hversu oft borðar þú að jafnaði saltkjötsbjúgu eða annað saltað/reykt kjöt?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í mánuði
- ☐ 1-3 í mánuði
- ☐ 1-2 í viku
- ☐ 3-6 í viku
- ☐ Daglega eða oftar

27. Hversu oft borðar þú að jafnaði saltfisk eða reyktan fisk?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í mánuði
- ☐ 1-3 í mánuði
- ☐ 1-2 í viku
- ☐ 3-6 í viku
- ☐ Daglega eða oftar

28. Hversu mikið salt notar þú í mat?

Merktu við þá fullyrðingu sem á best við um þig

- ☐ Ég strái yfirleitt salti á matinn minn áður en ég byrja að borða
- ☐ Ég salta stundum matinn minn á diskinn
- ☐ Ég salta sjaldan eða aldrei matinn minn á diskinn

29. Finnst þér yfirleitt matur sem þú færð í mötuneyti eða veitingahúsi...

- ☐ Ekki nægilega saltur
- ☐ Mátulega saltur
- ☐ Of saltur
- ☐ Fer sjaldan út að borða

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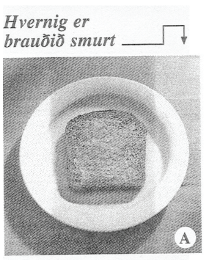
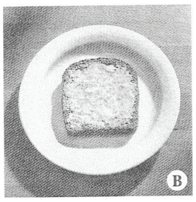
30. Hvernig smyrðu yfirleitt brauðið þitt?

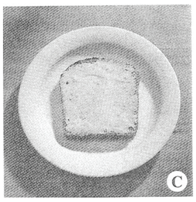
Á myndunum hér fyrir neðan eru brauðsneiðar með mismiklu smjöri/viðbiti

Merktu við þá mynd sem helst líkist því sem þú færð þér oftast.

- ☐ Mynd A lítið
- ☐ Mynd B í meðallagi
- ☐ Mynd C mikið
- ☐ Smyr yfirleitt ekki brauðið







Reyndu að rifja upp hvernig mataræði þitt var þegar þú varst á miðjum aldri, 40-50 ára:

31. Hversu oft borðaðir þú að jafnaði kjöt eða hakkrétti í aðalmáltíð þegar þú varst 40-50 ára?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

32. Hversu oft borðaðir þú að jafnaði fisk eða fiskrétti í aðalmáltíð þegar þú varst 40-50 ára?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

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33. Hversu oft borðarðir þú að jafnaði fiskiálegg, t.d. síld, sardínur, silung eða lax, á brauð eða í salat þegar þú varst 40-50 ára?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

34. Hversu oft borðaðir þú að jafnaði kartöflur þegar þú varst 40-50 ára?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

35. Hversu oft borðaðir þú að jafnaði ferska ávexti þegar þú varst 40-50 ára?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

36. Hversu oft borðaðir þú að jafnaði slátur, blóðmör eða lifrapylsu þegar þú varst 40-50 ára?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

37. Hversu oft borðaðir þú að jafnaði rúgbrauð, maltbrauð eða flatbrauð þegar þú varst 40-50 ára?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

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38. Hversu oft borðaðir þú að jafnaði heilhveitibrauð eða annað gróft brauð þegar þú varst 40-50 ára?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

39. Hversu oft borðaðir þú að jafnaði hafragraut eða múslí þegar þú varst 40-50 ára?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

40. Hversu oft borðaðir þú að jafnaði grænmeti þegar þú varst 40-50 ára?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

41. Hversu oft drakkst þú að jafnaði mjólk eða borðaðir mjólkurvörur þegar þú varst 40-50 ára?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

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42. Hvaða tegund af mjólk notaðir þú oftast/mest þegar þú varst 40-50 ára?

- ☐ Léttmjólk
- ☐ Nýmjólk
- ☐ Undanrennu
- ☐ Mysu
- ☐ Nota aldrei mjólk

43. Hversu oft tókst þú að jafnaði þorska- eða ufsalýsi eða lýsispillur (á samt ekki við lúðulýsispillur) þegar þú varst 40-50 ára?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega

44. Hversu oft borðaðir þú að jafnaði saltkjötsbjúgu eða annað saltað/reykt kjöt þegar þú varst 40-50 ára?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í mánuði
- ☐ 1-3 í mánuði
- ☐ 1-2 í viku
- ☐ 3-6 í viku
- ☐ Daglega eða oftar

45. Hversu oft borðaðir þú að jafnaði saltfisk eða reyktan fisk þegar þú varst 40-50 ára?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í mánuði
- ☐ 1-3 í mánuði
- ☐ 1-2 í viku
- ☐ 3-6 í viku
- ☐ Daglega eða oftar

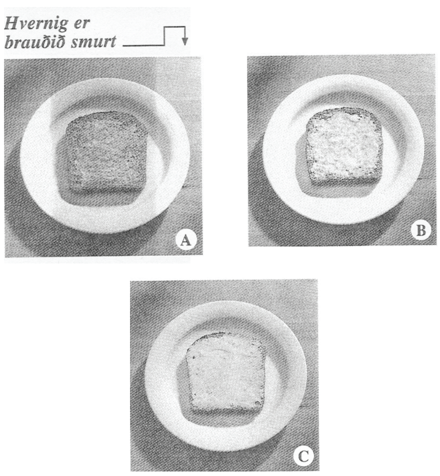
46. Hvaða tegund af viðbiti notaðir þú oftast/mest á brauð eða með mat þegar þú varst 40-50 ára?

- ☐ Borðsmjörlíki t.d sólblómasmjörlíki
- ☐ Venjulegt smjörlíki
- ☐ Smjör
- ☐ Notaði ekki viðbit

47. Hvernig smurðirðu yfirleitt brauðið þitt þegar þú varst 40-50 ára?

Skoðaðu myndirnar hér fyrir neðan og merktu við þá mynd sem helst líkist því sem þú fékkst þér oftast

- ☐ Mynd A lítið
- ☐ Mynd B í meðallagi
- ☐ Mynd C mikið
- ☐ Smurði yfirleitt ekki brauðið



Geturðu rifjað upp hvernig mataræði þitt var á unglingsárum 14-19 ára:

48. Hversu oft borðaðir þú að jafnaði kjöt eða hakkrétti aðalmáltíð þegar þú varst 14-19 ára?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni í dag

49. Hversu oft borðaðir þú að jafnaði fisk eða fiskrétti aðalmáltíð þegar þú varst 14-19 ára?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni í dag

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50. Hversu oft borðarðir þú að jafnaði fiskiálegg, t.d. síld, sardínur, silung eða lax, á brauð eða í salat þegar þú varst 14-19 ára?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

51. Hversu oft borðaðir þú að jafnaði kartöflur þegar þú varst 14-19 ára?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni í dag

52. Hversu oft borðaðir þú að jafnaði ferska ávexti þegar þú varst 14-19 ára?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni í dag

53. Hversu oft borðaðir þú að jafnaði slátur, blóðmör eða lifrarpylsu þegar þú varst 14-19 ára?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni í dag

54. Hversu oft borðaðir þú að jafnaði grænmeti þegar þú varst 14-19 ára?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í viku
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni í dag

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55. Hversu oft borðaðir þú að jafnaði saltkjötsbjúgu eða annað saltað/ reykt kjöt þegar þú varst 14-19 ára?

- [ ] Aldrei
- [ ] Sjaldnar en einu sinni í mánuði
- [ ] 1-3 í mánuði
- [ ] 1-2 í viku
- [ ] 3-6 í viku
- [ ] Daglega eða oftar

56. Hversu oft borðaðir þú að jafnaði saltfisk eða reyktan fisk þegar þú varst 14-19 ára?

- [ ] Aldrei
- [ ] Sjaldnar en einu sinni í mánuði
- [ ] 1-3 í mánuði
- [ ] 1-2 í viku
- [ ] 3-6 í viku
- [ ] Daglega eða oftar

57. Hversu oft borðaðir þú að jafnaði rúgbrauð, maltbrauð eða flatbrauð þegar þú varst 14-19 ára?

- [ ] Aldrei
- [ ] Sjaldnar en einu sinni í viku
- [ ] 1-2 í viku
- [ ] 3-4 í viku
- [ ] 5-6 í viku
- [ ] Daglega
- [ ] Oftar en einu sinni á dag

58. Hversu oft borðaðir þú að jafnaði hafragraut þegar þú varst 14-19 ára?

- [ ] Aldrei
- [ ] Sjaldnar en einu sinni í viku
- [ ] 1-2 í viku
- [ ] 3-4 í viku
- [ ] 5-6 í viku
- [ ] Daglega
- [ ] Oftar en einu sinni á dag

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59. Hversu oft tókst þú að jafnaði þorska- eða ufsalýsi eða lýsispillur (á samt ekki við lúðulýsispillur) þegar þú varst 14-19 ára?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í mánuði
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega

60. Hversu oft drakkst þú að jafnaði mjólk eða borðaðir mjólkurvörur þegar þú varst 14-19 ára?

- ☐ Aldrei
- ☐ Sjaldnar en einu sinni í mánuði
- ☐ 1-2 í viku
- ☐ 3-4 í viku
- ☐ 5-6 í viku
- ☐ Daglega
- ☐ Oftar en einu sinni á dag

61. Hvaða tegund af mjólk fékkst þú oftast/mest þegar þú varst 14-19 ára?

- ☐ Nýmjólk
- ☐ Undanrenna
- ☐ Mysu
- ☐ Rjóma/rjómabland
- ☐ Notaði aldrei mjólk

62. Hvaða viðbit fékkst þú oftast/mest á brauð eða með mat þegar þú varst 14-19 ára?

- ☐ Smjörlíki
- ☐ Smjör
- ☐ Bræðing
- ☐ Tólg/mör
- ☐ Notaði aldrei viðbit

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63. Hvernig smurðirðu yfirleitt brauðið þitt þegar þú varst 14-19 ára?

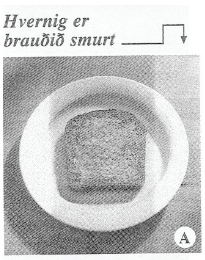
Skoðaðu myndirnar hér fyrir neðan og merktu við þá mynd sem helst líkist því sem þú fékkst þér oftast

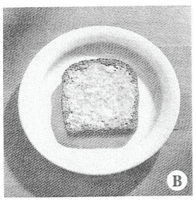
☐ Mynd A lítið

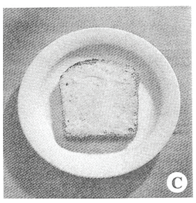
☐ Mynd B í meðallagi

☐ Mynd C mikið

☐ Smurði yfirleitt ekki brauðið







64. Veistu til þess að þú hafir fengið beinkröm í æsku?

(beinkröm er skortur á D-vítamíni og kemur fram sem mjúk og oft sveigð bein)

☐ Já

☐ Nei

☐ Veit ekki

65. Fékkstu alltaf nóg að borða þegar þú varst að alast upp?

☐ Fékk alltaf meira en nóg

☐ Fékk nóg, en ekkert umfram það

☐ Fékk stundum ekki nóg

☐ Var oft svöng/svangur í æsku

66. Ertu á sérstöku fæði nú?

☐ Já

☐ Nei → (Næst **69**)

☐ Veit ekki → (Næst **69**)