UiT The Arctic University of Norway

Faculty of Health Sciences

Department of Clinical Medicine

Vitamin D: Relations with Sleep and Bone Mineral Density

Insights from the Tromsø Study and randomized controlled trials.

Anette Uhlving Larsen

A dissertation for the degree of Philosophiae Doctor (PhD) – December 2021

Science, my boy, is composed of errors, but errors that it is right to make,
for they lead step by step towards the truth.

– Jules Verne

Table of Contents

Acknowledgements

The present work was carried out at UiT The Arctic University of Norway and the University Hospital of North Norway (UNN) in Tromsø from October 2015 to December 2021. I would like to thank UiT and the research program for the financial funding and for providing young students the opportunity to pursue their research ambitions early in the study course.

When I was accepted into the research program as a third-year medical student, I knew that joining an active research environment, while also learning the medical profession, would be challenging. Fortunately, I could not have been more privileged with my mentors. I would like to express my utmost gratitude to Guri Grimnes, for being willing to step up as my main supervisor from August 2020, during an otherwise extremely busy period of your career. With your kindness, optimism, and impressive effectiveness, you are a true inspiration. To my co-supervisor, Rolf Jorde, for taking me under your wing as a young student. You have been my oracle on this journey, and I will always admire your experienced scientific mind and genuine curiosity (and your endurance for hour-long walks in the mountains!). To my co-supervisor, Laila Hopstock, for sharing your expertise and insights on the Tromsø Study and for always providing me prompt and thorough feedback, despite your full-packed schedule.

This thesis would not have been possible without the selfless volunteering of the population of Tromsø; I am truly grateful for their willingness to participate in the studies. I also want to express my gratitude to the nurses at the Clinical Research Unit at UNN for their contribution with the clinical trials and to the Tromsø Study for use of data. To Yngve Figenschau and his staff at the Division of Diagnostic Services for excellent work with the laboratory analyses, Ole Martin Fuskevåg for the analysis of serum 25-hydroxyvitamin D, Børge Sivertsen and Sarah Cook for sharing the sleep duration and alcohol syntax files used in Paper I, Tom Wilsgaard for his input regarding the statistical analyses of Paper I-II, and Rod Wolstenholm for helping with my graphics in Paper III. To past and present members of the Endocrinology and Geriatric Research Group at the Department of Clinical Medicine, thank you for providing an inclusive environment with engaging scientific discussions and relaxing social events. To Cathrine and Malin, for the countless coffees, supportive talks, and late nights.

My heartly thanks go to all my friends and family, to mom and dad, for your unconditional love and endless support. Last but not least, to my best friend and most beloved fiancé, Sigurd, for bringing endless amounts of smiles and laughter to my life.

Norwegian summary/Norsk populærvitenskapelig sammendrag

I denne doktorgraden har sammenhengen mellom vitamin D og helserelaterte endepunkter blitt undersøkt.

I 2015-16 deltok mer enn 21,000 personer i Tromsøundersøkelsen. Deltakerne donerte blodprøver og svarte på spørreskjema om helsetilstanden, inkludert spørsmål om søvnhelsen. I dette materialet ble sammenhengen mellom vitamin D nivå og søvnlengde, trøtthet på dagtid og insomni (også kjent som søvnløshet) undersøkt. Det ble funnet en positiv sammenheng mellom vitamin D nivå og søvnlengde blant kvinner, samt lavere forekomst av insomni blant kvinner med normale vitamin D nivå sammenlignet med kvinner med høyt vitamin D nivå.

For å vite mer om denne sammenhengen, var det ønskelig å undersøke effekten av vitamin D tilskudd på søvnlengde, trøtthet på dagtid og forekomst av insomni. Det ble derfor inkludert et spørreskjema om søvnhelsen i en pågående intervensjonsstudie, som opprinnelig undersøkte effekten av vitamin D tilskudd i forebygging av hjerte- og karsykdom. Totalt 189 deltakere med lave vitamin D nivå ved studiestart fylte ut spørreskjemaet. Etter fire måneders behandling var det ingen forskjell i søvnlengde, trøtthet på dagtid eller forekomst av insomni mellom deltakere som hadde fått vitamin D og deltakere som hadde fått placebo (narremedisin). Det var heller ingen forskjeller stratifisert på kjønn, vitamin D nivå- eller søvnproblemer ved studiestart.

Videre ble det i en annen intervensjonsstudie undersøkt om vitamin D tilskudd uten tillegg av kalsium kunne ha en positiv virkning på beintettheten, som et forebyggende tiltak mot forringet beinhelse. I denne studien, som opprinnelig undersøkte om vitamin D kunne forebygge utvikling av type 2 diabetes, hadde vitamin D en positiv effekt på beintettheten hos menn, og effekten varierte avhengig av hvor beintettheten ble målt.

English summary

In this thesis, the relationship between vitamin D and health-related outcomes has been investigated.

More than 21,000 individuals participated in the Tromsø Study 2015-16. The participants donated blood samples and filled in questionnaires regarding their health status, including questions about their sleep health. Using these data, the association between vitamin D and sleep duration, daytime sleepiness, and insomnia (sleeplessness) were explored. In women, a positive association was found between vitamin D and sleep duration, while the prevalence of insomnia was lower in women with a normal vitamin D level compared to women with higher vitamin D levels.

To further explore this relationship, the effect of vitamin D supplementation on sleep duration, daytime sleepiness, and prevalence of insomnia was explored. A sleep questionnaire was implemented as part of an ongoing intervention study, originally designed to evaluate the effect of vitamin D supplementation for the prevention of cardiovascular disease. In total, 189 participants with low vitamin D levels at baseline filled in the sleep questionnaire. After four months of treatment, there were no differences in sleep duration, daytime sleepiness, or prevalence of insomnia between the group taking vitamin D versus placebo, neither when stratified by sex, nor by vitamin D level or sleep status at baseline.

It was further investigated in another intervention study whether vitamin D supplementation without additional calcium could have a positive effect on bone mineral density, as a preventive measure against deteriorated bone health. In this intervention study, originally designed to evaluate vitamin D for the prevention of type 2 diabetes, it was found that vitamin D supplementation alone had a positive effect on bone mineral density in men, and that the effect appeared to vary according to measurement site.

List of papers

Paper I.

Larsen AU, Hopstock LA, Jorde R, Grimnes G.

Vitamin D and sleep in the Tromsø study: Results from a population-based health survey.

Manuscript.

Paper II.

Larsen AU, Hopstock LA, Jorde R, Grimnes G.

No improvement of sleep from vitamin D supplementation: insights from a randomized controlled trial.

Sleep medicine: X 2021. 3:100040.

Paper III.

Larsen AU, Grimnes G, Jorde R.

The effect of high-dose vitamin D_3 supplementation on bone mineral density in subjects with prediabetes.

Osteoporos Int 2017. 29(1):171-180.

Abbreviations

ABCDEFGHIJKLMNOPQRSTUVWXYZ

25(OH)D	25-hydroxyvitamin D
$1,25(OH)_2D$	1,25-dihydroxyvitamin D, calcitriol
$24,25(OH)_2D$	24,25-dihydroxyvitamin D
BDI-II	Beck Depression Inventory II
BIS	Bergen insomnia scale
BMD	Bone mineral density
DBP	Vitamin D binding protein
DEQAS	Vitamin D external quality assurance scheme
DSM-5	Diagnostic and Statistical Manual for Mental Disorders, Fifth Edition
DXA	Dual-energy x-ray absorptiometry
EDS	Excessive daytime sleepiness
ESS	Epworth Sleepiness Scale
FGF23	Fibroblast growth factor 23
HbA1c	Glycated haemoglobin
ICSD-3	International Classification of Sleep Disorders, third ed.
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
ISD	Inadequate sleep duration
LC-MS/MS	Liquid chromatography tandem mass spectrometry
NHANES	National Health and Nutrition Examination Survey
OSA	Obstructive sleep apnoea
pQCT	peripheral quantitative computed tomography
PSQI	Pittsburgh Sleep Quality Index
PTH	Parathyroid hormone
RANKL	Receptor activator nuclear factor-κB ligand
RCT	Randomized controlled trial
RXR	Retinoid X receptor
SOL	Sleep onset latency
UV-B	Ultraviolet B
VDR	Vitamin D receptor

1 Introduction

The role of vitamin D in skeletal and extra-skeletal health has evolved substantially since its discovery in the early 1920s. It was long viewed as a fat-soluble vitamin only, essential in the preservation of calcium and phosphorus homeostasis (1). Today, vitamin D is recognized as a steroid prohormone that exerts a variety of functions through the vitamin D receptor (VDR) (1). The VDR is distributed in virtually all tissues throughout the body, and its discovery has substantiated the potential role of vitamin D in extra-skeletal health (2-4). Recently, a link between vitamin D and sleep has been highlighted, as low vitamin D levels are prevalent in populations with sleep complaints (5;6). A role of vitamin D in sleep is plausible considering that its metabolites are capable of crossing the blood brain barrier (7). Binding these metabolites, the VDR has been found in areas of the brain known to be involved in the regulation of sleep (8;9). Finally, the presence of key enzymes required for vitamin D metabolism have been demonstrated in brain cells (9-11), indicating local production and regulation in the brain. However, the available literature has to date provided inconclusive evidence, and so the role of vitamin D in the prevention and treatment of sleep disturbances remains unsettled.

The present thesis aimed to examine both skeletal and extra-skeletal effects of vitamin D. First, the association between vitamin D and self-reported sleep measures (including sleep duration, daytime sleepiness, and insomnia) was investigated in a general population living in the Arctic environment of Tromsø, Northern Norway (69° N). Second, using data from a previously performed randomized controlled trial (RCT), it was examined whether vitamin D supplementation could improve self-reported sleep measures (as described above) among individuals with low vitamin D levels. Finally, although vitamin D is undisputedly important in preventing bone mineralization deficits, there are still unanswered questions regarding its role in skeletal health. The direct effect of vitamin D supplementation (without additional calcium) on bone mineral density (BMD) remains controversial. Thus, by the use of a previously performed RCT, the effect of vitamin D supplementation on BMD was evaluated, and the results were included as part of this thesis elucidating the multiple effects of vitamin D.

1.1 Vitamin D

Vitamin D is a fat-soluble prohormone that has two main isoforms, namely vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol), which may both be obtained through the diet or via supplements. Vitamin D_2 is found primarily in mushrooms and yeast products, whereas vitamin D_3 is obtained from animal products such as cod liver oil, fatty fish, egg yolk and fortified dairy products (12). Vitamin D_3 may also be formed endogenously when the skin is exposed to ultraviolet (UV)-B rays. Initiated by wavelengths between ~280 to ~310 nm, a cholesterol derivate in the skin (7-dehydrocholesterol) is converted to previtamin D_3 through a photolytic process (1). Because previtamin D_3 is unstable at body temperature, it spontaneously forms vitamin D_3 through isomerization (13).

As there are few dietary sources, endogenous production in the skin constitutes the most important source of vitamin D. However, dermal synthesis depends on UV-B availability, which is affected by latitude, season, and time of the day (14). In Tromsø (69° N), the period with sufficient UV-B radiation for vitamin D synthesis stretches from March to September and is often compromised by the presence of cloudy weather (15). Nutritional and behavioural factors may reduce or amplify the impact of season and latitude (16). Thus, intake of traditional marine foods and/or use of vitamin D supplements, sunbeds, sunscreen, choice of clothing, travelling on sunny holidays and other sun-seeking habits may influence the vitamin D status of an individual. Other important determinants of vitamin D status include body composition, sex, age (17), genetic factors (18), and skin pigmentation (16), as well as medication use and various conditions altering absorption and/or metabolism of vitamin D (19).

Whether absorbed in the gut or produced in the skin, vitamin D ultimately reaches the circulation, where it is transported primarily to the liver. By actions of hepatic 25-hydroxylase enzymes, vitamin D is hydroxylated to form the major circulating metabolites 25-hydroxyvitamin D_2 ($25(OH)D_2$) and D_3 ($25(OH)D_3$) formed from vitamin D_2 and D_3, respectively. In the following, the two will be collectively referred to as 25(OH)D (unless distinction is important) as they share similar metabolism. The formation of this inactive metabolite is not strictly regulated, and so 25(OH)D reflects vitamin D obtained from all sources. With a relatively long half-life (of 2-3 weeks) and high serum concentration, total s-25(OH)D is considered the most reliable biochemical marker to evaluate an individual's vitamin D status (20).

The active hormone 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) is formed through hydroxylation of 25(OH)D (21). This process mainly takes place in the kidneys by actions of the 1α-hydroxylase enzyme (CYP27B1), although the expression of CYP27B1 has also been demonstrated in extrarenal sites (21). Renal activation of vitamin D is stringently controlled by regulators of calcium and phosphate metabolism, including parathyroid hormone (PTH) and fibroblast growth factor-23 (FGF23). In short, PTH increases renal formation of $1,25(OH)_2D$ by upregulating CYP27B1 activity in response to low serum calcium and high serum phosphate concentrations. Likewise, high concentrations of serum calcium and $1,25(OH)_2D$ down-regulate PTH activity. FGF23 activity is stimulated by elevated levels of serum phosphate and $1,25(OH)_2D$; Cooperating with the transmembrane protein αKlotho, FGF23 supresses CYP27B1 activity and promotes the catabolizing 24-hydroxylase enzyme (CYP24A1). The activity of CYP24A1 is also highly regulated, as it decreases $1,25(OH)_2D$ levels by converting both 25(OH)D and $1,25(OH)_2D$ to inactive metabolites. These metabolites are then excreted with the bile and urine (21). The synthesis, metabolism and activation of vitamin D is summarized in **Figure 1**, along with the biological effects of $1,25(OH)_2D$ on calcium, phosphorus, and bone metabolism.

Activated $1,25(OH)_2D$ exerts its functions through binding of the VDR in the cell nucleus. This implies that the $1,25(OH)_2D$ must either cross the cell membrane or be produced within the cell. When in the circulation, the vitamin D metabolites are mainly bound to vitamin D binding protein (DBP) (~85%), with a smaller fraction bound to albumin and lipoproteins (~15%) (22). Less than 1% circulate in the free fat-soluble form, which presumably diffuse passively across the cell membranes. According to the free hormone hypothesis, it is the unbound (free) fraction of vitamin D that may enter the cells (21). However, vitamin D may also enter the cells via megalin- and cubilin-mediated endocytosis. Through this mechanism transmembrane receptor proteins (i.e., megalin and cubilin) may internalize the entire vitamin D metabolite/DBP complex (21). Traditionally, this has been considered of particular importance in renal uptake and metabolism of 25(OH)D (23), although expression of these endocytic receptors have also been demonstrated in various extrarenal tissues (24). The importance of this mechanism in extrarenal tissues has not been settled (21;25).

Upon binding, $1,25(OH)_2D$ induces a conformational change to the VDR, facilitating interaction with the retinoid X receptor (RXR). This coregulatory protein is required for the $1,25(OH)_2D$/VDR complex to act as a transcription factor (21). The activated complex may then attach to specific DNA sequences, namely vitamin D responsive elements (VDREs), in

and around vitamin D target genes to up- or down-regulate the transcription of these genes (21). Thus, activated $1,25(OH)_2D$ may exert a wide array of pleiotropic effects through the VDR. In fact, the expression of this receptor, along with key enzymes required for vitamin D metabolism and activation, has been demonstrated not only in target cells of enterocytes, liver, kidney, and bone, but in an extensive number of tissues, suggesting a role of vitamin D beyond maintenance of calcium and phosphorus homeostasis (4).

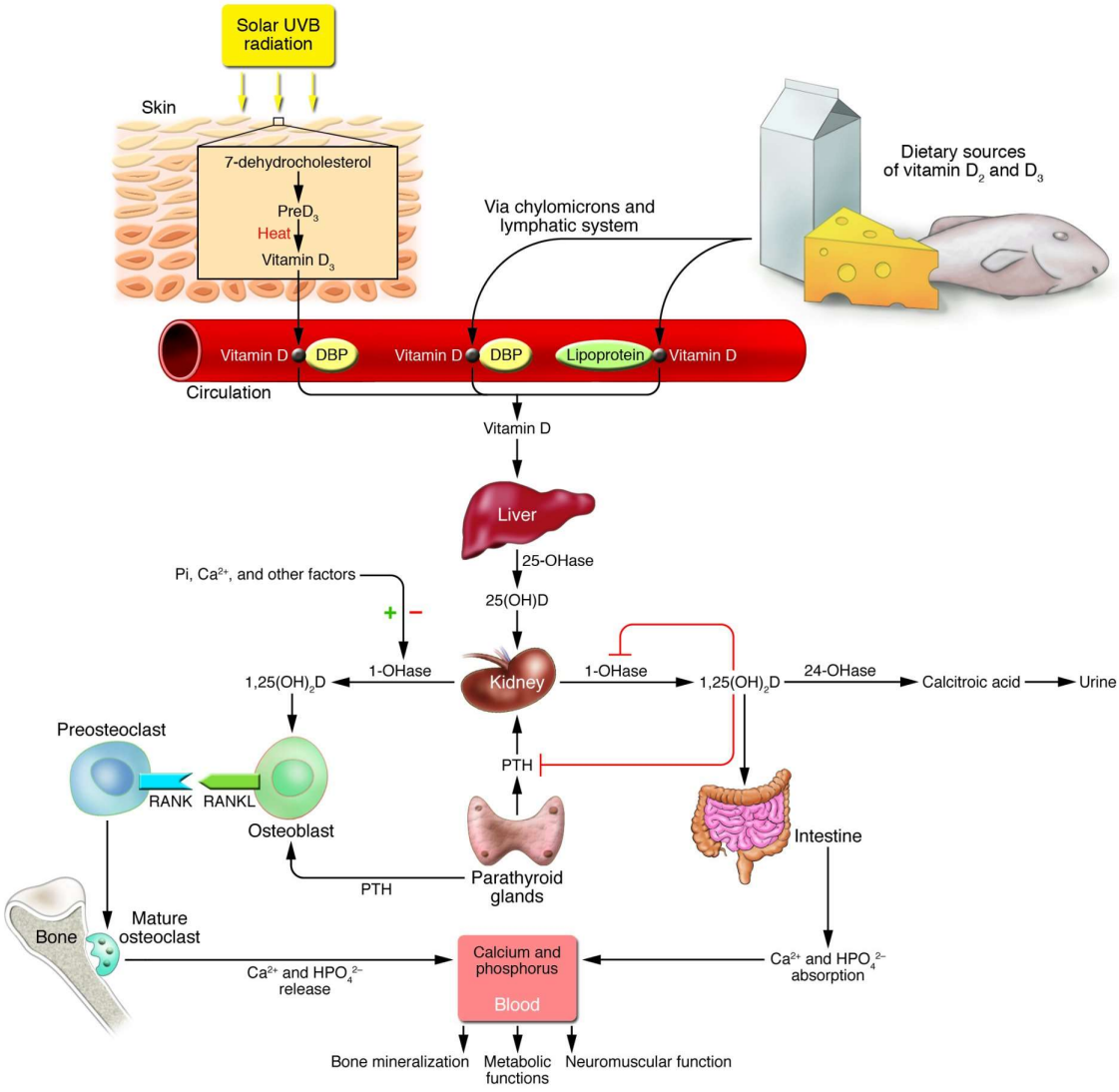


Figure 1. The synthesis, metabolism and activation of vitamin D, and the biologic effects of $1,25(OH)_2D$ on calcium, phosphorus and bone metabolism (12). Used with permission from the American Society for Clinical Investigation and Copyright Clearance Center.

1.1.1 Vitamin D measurements

As mentioned, total s-25(OH)D (the sum of free and protein bound 25(OH)D) is considered the most reliable biochemical marker of an individual's vitamin D status (20). Unless otherwise specified, total s-25(OH)D will be referred to as simply s-25(OH)D in the following. Historically, efforts to define an optimal vitamin D status have been compromised by significant variation in s-25(OH)D assays between studies (26). The most accurate and precise method for measuring s-25(OH)D is liquid chromatography-tandem mass spectrometry (LC-MS/MS), separating total s-25(OH)D from other vitamin D metabolites (20). However, the method requires trained personnel and expensive equipment, which is why the use of less precise automated immunoassays remains common. Thus, vitamin D standardization programs, including the Vitamin D external Quality Assessment Scheme (DEQAS) (27) and the Vitamin D Standardization Program (VDSP) (26), have been recommended for standardizing s-25(OH)D measurements in research, as well as in the clinical setting (20).

1.1.2 Vitamin D status

To date, there is no firmly established international consensus on how to define an inadequate vitamin D status. Most recommendations state that values of s-25(OH)D below 30 nmol/L (< 12 ng/ml) should be recognized as severe vitamin D deficiency due to the increased risk of rickets (children) and osteomalacia (adults) (20;28). For skeletal integrity, levels between 50-125 nmol/L (20-50 ng/ml) are considered safe and sufficient in the general population (20). However, benefit of higher thresholds has been suggested, along with a cut-off value above 75 nmol/L (> 30 ng/ml), for optimal risk reduction in extra-skeletal outcomes (28).

Using standardized data from epidemiological studies, the ODIN study has estimated the prevalence of severe vitamin D deficiency (s-25(OH)D < 30 nmol/L) in Europe to about 12% (29). Despite shorter seasons of sufficient UV-B exposure, estimates in Northern Europe have been lower (30). Presumably, the prevalent use of food fortification programs, in combination with a high intake of traditional marine foods, use of cod liver oil and vitamin D promoting sun-seeking habits (as previously described) reduces the impact of season and latitude on vitamin D status in the North (30;31). In Norway, vitamin D deficiency is particularly common among immigrants (32), adolescents (33;34) and older adults (nursing home residents in particular) (35).

To maintain a vitamin D status above 50 nmol/L (20 ng/ml), supplementation is required in most individuals (28). Although there is currently no international consensus (36), doses from 400-2000 IU/day (10-50 μg) are considered safe and commonly available (28;31). In Norway, the recommended daily intake of vitamin D in the general population is 400 IU/day (10 μg) for children and adults, and 800 IU/day (20 μg) for individuals of 75 years or older, according to the Nordic nutrition recommendations (37). Supplementation regimens of longer dosing intervals using bolus doses are not recommended and have been associated with increased risk of falls and fractures (38). However, rapid improvement of vitamin D levels may be necessary (such as in the critical care setting), in which an initial loading dose followed by daily dosing may be required (39). Still, the safe upper limit of vitamin D is yet to be established. Hypercalciuria, hypercalcemia and hyperphosphatemia are considered hallmarks of vitamin D toxicity, and may cause calcification of soft tissue, formation of kidney stones, disturbance of other electrolytes and arrhythmias (40). Vitamin D intoxication does not occur from UV-exposure alone due to autoregulation in the skin when exposed to excessive sunlight (40), and rarely arises from s-25(OH)D below 375 nmol/L (150 ng/ml) (31). Although doses up to 10,000 IU/day (250 μg) has been reported as safe by the Endocrine Society (41), such amounts are rarely required in the clinical setting (28). Moreover, as data from observational studies have suggested a U-shaped dose-response curve for the association between s-25(OH)D and mortality (42), the Institute of Medicine (IOM) and The European Food and Safety Authority both recommend staying below 4000 IU/day (10 μg) in otherwise healthy individuals (43;44).

1.2 Sleep

Sleep is a daily biological imperative in almost all living species. As humans, we spend one third of our lifetime in this reversible physiological state of reduced responsiveness (45). The importance of sufficient amount and quality of sleep has been underscored by the serious adverse health effects associated with sleep problems, including impaired daytime function (46) and alterations of both mental (47;48) and physical (49) health and well-being.

The amount of sleep that is considered sufficient varies throughout the life span (50). Adults between 18 and 60 years of age are recommended a minimum of seven hours of sleep per night on a regular basis for optimal sleep health (50;51). The proportion of adults not reaching this goal is worryingly high (52;53), and insufficient sleep is increasingly being recognized as a public health concern worldwide (54-56). In the US, costs related to insomnia alone have

been estimated to over 90 billion USD a year, including both direct medical costs, and indirect costs related to accidents and lost work capacity (57). Estimates are currently not available in Norway, but previous studies have linked poor sleep to lower school performance (58), and higher risk of school absence (59), sick leave (60) and work disability (61). This is concerning, as both insufficient sleep and insomnia are highly prevalent conditions seemingly on the rise in Norway (62;63).

Sleep disturbances are particularly frequent in the elderly, as well as in teenagers (45). It has been argued that sleep traits in older adults change due to factors accompanying age rather than age itself (e.g., increased risk of illnesses and need of medications) (64). Nevertheless, alterations of sleep duration and quality have been shown to change with age independent of these factors (65;66). The risk of sleep disturbances is also influenced by sex (67). Women are especially prone to sleep disturbances during pregnancy and perimenopause (45), and have as much as 40% higher odds of insomnia throughout their lifetime compared to men (67). The relationship between sex and sleep has also been described as confusing (45), as women with self-reported insomnia show no indications of objectively measured short sleep duration or altered sleep architecture (45;68). In addition to age and sex, other major risk factors have been described, such as having shift work, experiencing stress due to work or family life (45;69) or high alcohol consumption (70). The role of additional factors such as environmental noise and light pollution, use of electronic devices, diet, and other lifestyle related factors is yet to be fully elucidated (45).

The pathophysiology of sleep disorders is complex and only partly understood, and the mechanisms involved are likely multifactorial. Although variation in both quality and quantity of sleep is an unavoidable part of life (at least to some degree), sleep disturbances that cause serious daytime dysfunction and persist over time, may be classified as a sleep disorder. Classification of sleep disorders are described in the 3rd edition of the International Classification of Sleep Disorders (ICSD-3) by the American Academy of Sleep Medicine (71) and categorized into the following sections: 1) insomnia; 2) sleep related breathing disorder; 3) central disorders of hypersomnolence; 4) circadian rhythm sleep-wake disorders; 5) parasomnias; 6) sleep related movement disorders; and 7) other sleep disorders. Another important classification is found in the 5th edition of the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSM-5) (72). Despite minor discrepancies between the DSM-5 and the ICSD-3 (73), these differences do not have implications for the present thesis and will therefore not be further elaborated.

1.3 Measuring sleep

There are several dimensions used to described sleep, including sleep duration, -timing, -continuity, and -architecture. Sleep quality is also frequently reported, although there is no international consensus on how to define this construct (74). As a measure of subjective sleep health, sleep quality often refers to the perception of having slept well, usually characterized by short sleep onset latency (SOL), uninterrupted night-time sleep, and a feeling of being energized and rested the following day (75).

Due to its ability to describe sleep architecture (i.e., modulation of sleep stages during a sleep period), PSG is most often referred to as the gold standard of sleep recordings (76). PSG require expensive equipment and experienced personnel to interpret the results, which is often a challenge in low-resource settings. Another problem is the method's intrusiveness, as both the unfamiliar environment in the sleep laboratory and the discomfort of the electrodes may influence the sleep recording. For instance, individuals tend to sleep worse during the first night of PSG (known as the "first-night effect") (77).

The need for cheaper, less intrusive methods has contributed to the increased use of accelerometery, often called actigraphy. Actigraphic methods are suitable for longer recordings in natural environments and may capture night-to-night variability in sleep. In the last few years, the development of consumer-based activity trackers that also aim to measure sleep (such as fitness trackers and smartwatches) have been considerable (78). These often low-cost and non-invasive devices include various sensor technology, typically including an accelerometer (to detect changes in movement) and photoplethysmography (to detect changes in pulse). In combination, these devices may provide proxy-measurements of sleep and have also been used in research. However, lack of consensus regarding instrumentation, algorithms, software, and reporting, represents significant challenges in actigraphic recording; Different procedures may provide significant differences in estimated sleep parameters, even when recommended procedures are followed (79).

Finally, both qualitative and quantitative aspects of sleep can be measured using self-report. Not only are self-reported sleep measures easily accessible, but they also have the advantage of being perceived as the most important by the individual (45). Comparisons of self-reported and objective sleep measures have provided conflicting evidence; Whereas some have found an adequate correlation (74;80;81), others have reported discrepancies between subjective and

objective sleep measures (68;82). Correlations between self-reported sleep duration and actigraphic and polysomnographic measures have been reported as moderate in recent large cohort studies (83-85).

In the present thesis, when referred to "self-reported sleep measures" this includes self-reported measures of sleep duration, daytime sleepiness, and insomnia (as described in detail in section 3.2.4).

1.4 Vitamin D and sleep

The newfound link between vitamin D and sleep disturbances is biologically plausible (5;6). As a fat-soluble steroid, vitamin D is capable of crossing the blood brain barrier (7) and both 25(OH)D and $1,25(OH)_2D$ have been demonstrated in the human brain (86;87). Brain cells also express key enzymes required for vitamin D metabolism (CYP2R1) (10), activation (CYP27B1) (9), and degradation (CYP24A1) (11), indicating local production and regulation of vitamin D. Expression varies according to site (9) and type of brain cell (11), and the production of transcripts coding for these enzymes is small relative to that of the liver and kidneys (11). Thus, vitamin D likely serves auto- and/or paracrine purposes in the brain. The expression of CYP27B1 in cells lacking VDRs further indicates a paracrine, rather than autocrine mode of action (9). Consolidating a role of vitamin D as a neurosteroid, animal studies have identified target cells of vitamin D in areas of the brain and brainstem that home pacemaker cells – essential in the early stages and maintenance of sleep (88-90). The widespread, but distinctive distribution of the VDR found in rodents, have been described as strikingly similar in humans (9). In particular, immune-histochemical studies using VDR-binding antibodies have demonstrated the presence of VDRs in the hypothalamus (9;91) and substantia nigra (9;92). Interestingly, these areas also strongly express the activating enzyme CYP27B1 and are known to be involved in the initiation, maintenance, and timing of sleep (8;9).

To date, the detailed actions of vitamin D involved in sleep regulation have not been fully elucidated, but several mechanisms have been suggested (93;94). The circadian clockwork in mammals is a complex system comprised of a central pacemaker located in the suprachiasmatic nuclei (SCN) of the hypothalamus, and in subsidiary "clocks" present in virtually all cells. Vitamin D has been suggested an important role in regulating the molecular clock, as $1,25(OH)_2D$ is able to synchronize the expression of circadian clock genes in

adipose-derived stem cells (95). The central clock is synchronized daily to the 24-hour light/dark cycle, as specialized photoreceptors in the retina (hereby referred to as retinal ganglion cells) perceive photic signals and transmit these to the SCN neurons (96). In response to these light signals, the SCN inhibits formation and release of the sleep hormone melatonin from the pineal gland. Interestingly, vitamin D deficiency has been associated with loss of visual axons and retinal ganglion cells (97), which could ultimately increase melatonin levels (96;98). In contrast, another theory has suggested that vitamin D may indirectly increase melatonin production by inducing the tryptophan hydroxylase (TPH)-2. The TPH-2 enzyme converts tryptophan to 5-hydroxytryptophan, stimulating the formation of serotonin – the main substrate for melatonin synthesis (99). However, the majority of tryptophan (>95%) is catabolized by other tryptophan-converting enzymes; Via the kynurenine pathway, tryptophan is converted to kynurenine and other inflammatory metabolites (100). Sleep deprivation increases inflammatory signalling and the release of stress hormones, which triggers the activation of enzymes in the kynurenine pathway (100). Thus, by diverging tryptophan towards the serotonin-pathway vitamin D might counteract the effects of sleep deprivation by reducing levels of kynurenine and other inflammatory mediators.

The anti-inflammatory properties of vitamin D and its role as an important immune modulator has been increasingly recognized (101). In relation to sleep research, vitamin D has been shown to suppress the formation of interleukin (IL)-6 (102), a key inflammatory marker associated with increased risk of obstructive sleep apnoea (OSA) (103). Elevated levels of IL-6 have also been associated with increased pain sensation related to sleep deprivation (104). Interestingly, supplementation with vitamin D has been reported to relieve both pain symptoms and to improve sleep quality (105;106). However, methodological concerns, including small sample sizes (105;106), lack of a proper control group (105) and coadministration of anti-depressive medication (106), prevails firm conclusions based on these studies. Thus, it remains unclear whether vitamin D may represent an indirect role to improve sleep by alleviating pain symptoms, and/or share interrelated pathways in the regulatory mechanisms of sleep and pain.

Numerous large population studies have examined the association between vitamin D and sleep; A recent meta-analysis (107), including six cross-sectional studies (108-113), two case-control studies (114;115) and one cohort study (116), reported that vitamin D deficiency (< 20 ng/ml or < 50 nmol/L) was associated with increased odds of sleep disorders. Several studies from the Korean National Health and Nutrition Examination Survey (KNHANES) have

reported an association between vitamin D deficiency and short sleep duration (117-119), although a recent study using data from the 2010-2012 cohort was not able to reproduce this finding (120). Another study, using data from the NHANES in the US, reported that low vitamin D levels were associated with longer time to fall asleep, but found no association with sleep duration (121).

The association between vitamin D and sleep has been reported in specific populations with contrasting results; For instance, vitamin D deficiency has been correlated with poor sleep quality in first-trimester pregnant women (116), whereas no relationship was found in a different study including last-trimester pregnant women (111). In haemodialysis patients, vitamin D deficiency has been associated with poor sleep quality (122;123), but not with excessive daytime sleepiness (EDS) (124). It is likely that variations in methodology, such as the selection of covariates/adjusted factors, measuring scales and outcomes, as well as the populations examined, may have contributed to the observed discrepancies.

Few clinical trials have examined the effect of vitamin D supplementation on sleep. The effect of dietary supplements in general were recently summarized in a systematic review and meta-analysis of RCTs, concluding that vitamin D could improve subjective sleep quality (125). Among the four RCTs including data on vitamin D supplements (106;126-128), three studies (106;126;127) reported an improvement of subjective sleep quality in the group taking vitamin D supplements (pooled MD -1.63, 95% CI -3.15 to -0.10) (125). However, heterogeneity between the included studies was high (I^2=85%), and the fourth study reported that repletion of s-25(OH)D (≥ 32 ng/L or ≥ 80 nmol/L) resulted in an overall worse sleep quality compared to those who remained insufficient (128). Thus, the association between vitamin D and sleep, and the potential role of vitamin D as a simple, safe, and inexpensive intervention to improve sleep health, is yet to be firmly established.

1.5 Bone Metabolism

The skeleton provides structural support and protection of internal organs and facilitates movement of the body. Constituted by a central marrow space surrounded by bone tissue and periosteum, the bones both serve as a storage of minerals and growth factors, and as an active metabolic tissue that regulates mineral and acid-base homeostasis (129). The periosteum provides blood and nerve supply to the bones, as well as an attachment site for tendons and ligaments, whereas the bone marrow is an important site for haematopoietic activity (129;130).

Structurally, bone is divided into cortical (compact) and trabecular (cancellous) bone. The two types share similar matrix composition, but differ in site, density, three dimensional structure, and metabolic activity (131). Cortical bone is found especially in the diaphysis (or shaft) of long bones and on the surface of flat bones, whereas trabecular bone is found in the metaphysis (or end) of long bones, as well as in the vertebrae, pelvic, and inner part of flat bones (132). The major structural unit of cortical bone are the cortical osteons, which consist of longitudinal cylinders that run in parallel to the long bone axis, providing compressive strength to the skeleton (132). Trabecular bone is considerably less dense, which allows deformation and absorption of loads in response to mechanical stimuli (132). Although constituting only 20% of the total weight of the skeleton, trabecular bone has a significantly larger surface area per unit volume compared to cortical bone (132). Its trabecular arrangement maximizes nutrient diffusion and ensures exposure of trabecular bone cells to circulating cytokines, growth factors and other hormones. This is crucial for the ability of trabecular bone to maintain metabolic activity and bone remodeling (131;132). The three-dimensional structure of bone is illustrated in **Figure 2**.

Adequate mineralization is important to maintain structural integrity of the skeleton and is achieved by crystalizing circulating calcium and phosphate within the organic bone matrix. Mature, calcified bone contains about 20-25% organic matrix (made up by type I collagen (>90%), bone cells, proteoglycans, glycoproteins, and growth factors). In addition, inorganic minerals (mainly hydroxyapatite) constitute about 65-70% of calcified bone and a minor fraction is made up by water and lipids (131;133).

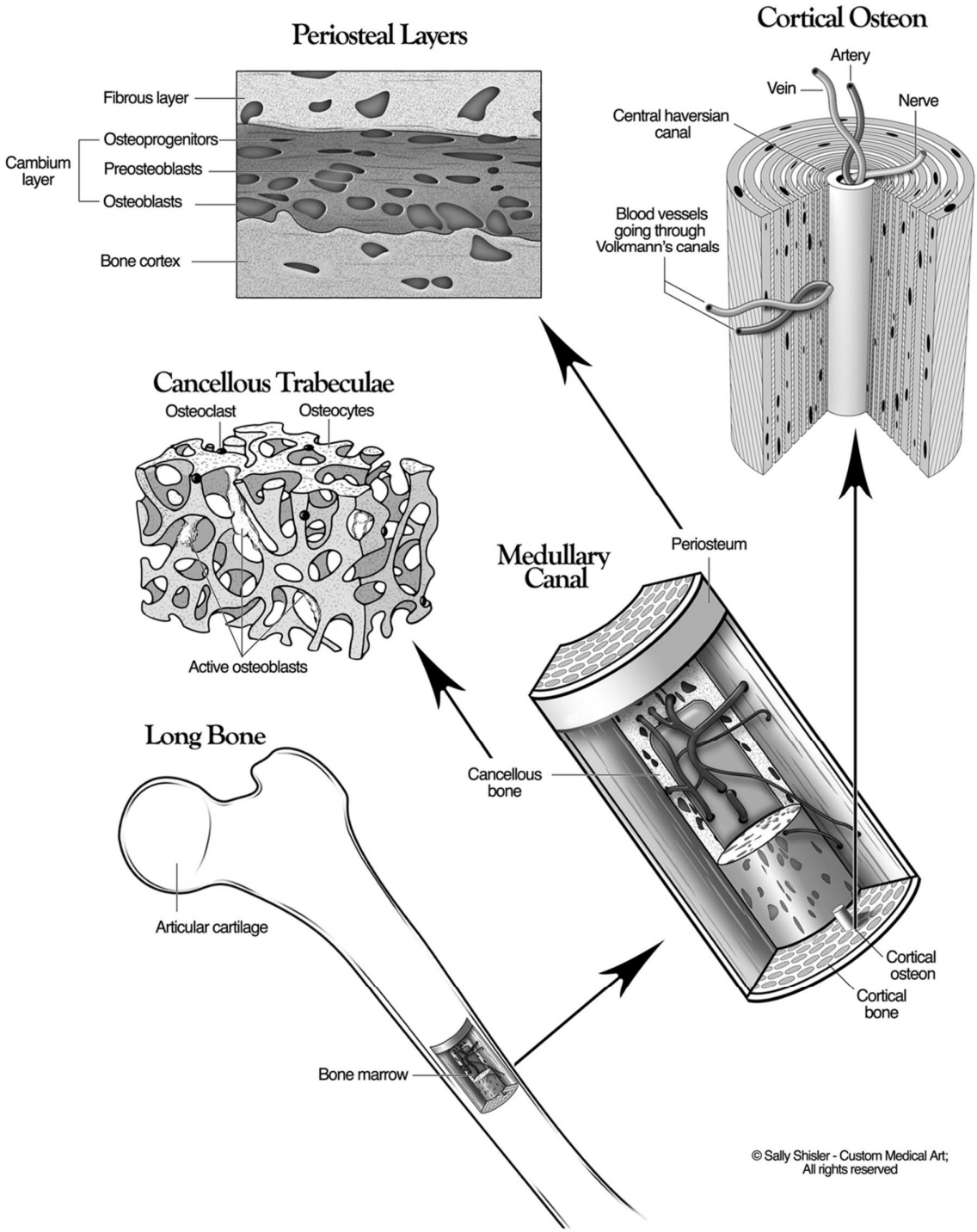


Figure 2. Three-dimensional structure of bone (131). Reused with the permission from Wolters Kluwer Health, Inc. and Copyright Clearance Center.

The three main types of bone cells constitute about 10% of the total bone volume (131). These include osteoclasts (derived from the hematopoietic stem cells), osteoblasts and osteocytes (both derived from the mesenchymal stem cells) (132). An osteoclast is a large, multinucleated cell that is responsible for bone resorption of mineralized matrix, whereas an osteoblast is a bone-forming cell that synthesize and secrete organic bone matrix, as well as regulating its mineralization (132). Once activated, the osteoblast may remain quiescent (serving as a protective lining on the bone surface), further develop into an osteocyte or undergo apoptosis (132;134). Osteocytes constitutes about 90% of all bone cells and may be described as specialized, fully differentiated osteoblasts that are surrounded by their own calcified matrix (132). The osteocytes functions as orchestrators of bone deposition and resorption and influences the activity of osteoblasts and osteoclasts in response to mechanic stress signals (129;131).

Bone strength and mineral homeostasis is sustained through bone remodelling, in which bone resorption by the osteoclast is tightly balanced against bone formation by the osteoblast (129). As a continuous process that occurs throughout the lifetime, bone remodelling results in complete renewal of the skeleton every ten years (134). The process of bone remodelling is believed orchestrated by osteocytes, defining when and where remodelling should take place in response to endocrine, neuronal and local factors (133;135). Crosstalk between the different bone cells facilitate assembling of osteoblasts only at sites where osteoclasts have recently completed resorption (134). The coupling signals demarcating the end of bone resorption and the beginning of bone formation are not yet fully understood (129).

Highly relevant in the context of bone remodelling is the interaction between two transmembranous proteins; In response to inflammation and activation of T-cells, the receptor activator nuclear factor κappa B (RANK) ligand, expressed primarily by osteoblasts, interacts with its RANK receptor, expressed on osteoclast precursor cells (and on mature osteoclasts). Through a cascade of events, the interaction ultimately causes activation of osteoclasts, thereby increasing bone resorption (133;134). This crosstalk mechanism between osteoblasts and osteoclasts is strictly regulated. Osteoprotegerin (OPG), secreted mainly by osteoblasts, acts as a competitive binding partner for RANK-ligand and inhibits its interaction with RANK (134;136). Thus, the RANK-ligand/OPG ratio serves as an important regulator of osteoclastogenesis and bone resorption. The RANK-ligand is also targeted by calciotropic hormones (i.e., stimulators of osteoclastogenesis), including $1,25(OH)_2D$, PTH and calcitonin in particular (133).

Bone mass increases throughout childhood and adolescence, until a peak is reached in early adult life (132;133). Thereafter, bone mass slowly declines, as balanced remodelling minimizes net loss until menopause in women and until 60 years in men, a point from which loss of cortical bone mass accelerates (137;138). Trabecular bone loss increases from earlier age in both sexes, but also accelerates during perimenopause in women (138). The accelerated post-menopausal decline in bone mass is first and foremost caused by altered hormonal status (falling levels of estrogen in particular) and older age (139). Estrogen have antiapoptotic effects in osteoblasts and osteocytes, but proapoptotic effects in osteoclasts (140;141). Thus, falling estrogen levels increase bone resorption by the osteoclast. Other major determinants of age-related bone loss include decreased physical activity (leading to reduced mechanical load) and alteration of nutritional status, including vitamin D deficiency, decreased calcium absorption from the gut and reabsorption in the kidneys (139). Also, long-term use of glucocorticoids, smoking and alcohol intake, all contribute to lower bone mineral content and altered bone strength (139).

Bone loss significantly increases bone frailty and porosity, and more so in women than in men. Women obtain lower peak bone mass in early life and experience larger age-related bone loss due to hormonal changes during menopause (139). According to guidelines for the prevention and treatment of osteoporosis (142;143), men aged 50 years or older and postmenopausal women regardless of age may be diagnosed with osteoporosis if any of the following criteria is met: 1) BMD assessed with dual-energy x-ray absorptiometry (DXA) at the lumbar spine, femoral neck or total hip indicating a T-score $\leq$ -2.5 standard deviations of the mean of healthy young women, 2) low-trauma spine or hip fracture (regardless of BMD), 3) low-trauma fracture of the proximal humerus, pelvis and/or distal forearm in the setting of low bone mass (osteopenia) confirmed by DXA (T-score between > -2.5 and < -1.0) and/or 4) increased fracture probability assessed using the Fracture Risk Assessment tool.

1.6 Vitamin D and Bone Mineral Density

The close connection between vitamin D and bone metabolism is widely acknowledged. Vitamin D deficiency may cause rickets in children and osteomalacia in adults (1), and may accelerate bone loss and osteoporosis in the elderly (3). Some of the main purposes of vitamin D is to maintain concentrations of calcium and phosphate in the blood, and to prevent secondary hyperparathyroidism. Blood calcium concentrations are tightly monitored by calcium-sensing transmembrane proteins in the parathyroid glands. PTH is released in

response to low levels of calcium and $1,25(OH)_2D$ and high levels of phosphate (144). By increasing the formation of $1,25(OH)_2D$ in the kidneys, PTH stimulates intestinal calcium absorption and increases urinary output of phosphate (144). If this fails to increase calcium levels in the blood (for instance due to inadequate dietary intake), the continued secretion of PTH and formation of $1,25(OH)_2D$ also stimulate reabsorption of filtered calcium in the distal renal tubule (21). In the absence of adequate calcium supply, $1,25(OH)_2D$ also sustain calcium concentrations at the expense of internal stores by stimulating bone resorption (21). Thus, first line therapy for people at risk of osteoporotic fractures is vitamin D together with calcium (145).

Traditionally, $1,25(OH)_2D$ has been considered to influence bone mineralization indirectly through its role in maintaining calcium and phosphorus homeostasis. However, emerging evidence suggest that $1,25(OH)_2D$ may also influence bone cells directly (146;147). When calcium concentrations are low, $1,25(OH)_2D$ stimulates the expression of RANK ligand in osteoblasts through binding of the VDR (21). Combined with an increased level of potent inhibitors of mineralization (such as pyrophosphate and osteopontin), $1,25(OH)_2D$ enhances osteoclastogenesis and reduces mineralization (21). Thus, when calcium concentrations are low, the actions of $1,25(OH)_2D$ are directed to preserve serum calcium at the expense of bone. The actions of $1,25(OH)_2D$ in bone cells during normal or positive calcium balance are not yet fully understood, but likely varies depending on the osteoblast differentiation stage: In osteoprogenitors and young osteoblasts, VDR signalling negatively regulates bone mass through increased expression of RANK ligand (stimulating osteoclastogenesis and bone resorption). In contrast, binding of VDR in the mature osteoblast may 1) decrease RANK ligand/OPG ratio, inhibit osteoclastogenesis, and 2) increase expression of genes mediating anabolic effects in osteoblasts, ultimately increasing bone mass (21). Finally, in osteocytes (the final step of osteoblast differentiation), VDR signalling is probably redundant for bone metabolism (148). Thus, depending on the physiological and pathological circumstance, vitamin D may positively or negatively regulate bone mass and bone mineral content (147). The exact role of these differential effects of VDR signalling in bone cells during normal and positive calcium balance is not yet fully elucidated and requires further investigation.

A vast number of observational studies have investigated the relationship between vitamin D and BMD. In general, increased s-25(OH)D concentrations and/or increased intake of vitamin D is found to positively predict BMD (149). Concerns regarding a U-shaped relationship have

also been raised, as s-25(OH)D concentrations > 100 nmol/L (> 40 ng/mL) have been inversely associated with BMD (150).

A substantial selection of systematic reviews and meta-analyses have summarized the results of previous RCTs evaluating the effect of vitamin D on musculoskeletal outcomes, including BMD (151-158). In the most recent meta-analysis (156), vitamin D supplementation alone resulted in an increase in BMD at several measurement sites, including 0.34% at the total hip (95%CI 0.13-0.55), 0.76% at the femoral neck (95%CI 0.42-1.09), and 0.25% at the lumbar spine (95%CI 0.00-0.49) compared to placebo. Given a prespecified futility boundary to identify clinically relevant effects (being set to 0.5% at the total hip, forearm and total body BMD, and to 1.0% for the lumbar spine and femoral neck) the authors concluded that vitamin D supplementation did not "have meaningful effects on BMD" (156). However, the majority of the included RCTs were performed in women aged >65 years and the trials results were not compared by sex.

2 Aims and objectives of the thesis

1. To examine the association between s-25(OH)D and self-reported sleep measures, including sleep duration, daytime sleepiness, and insomnia.
2. To examine the effect of vitamin D supplementation on self-reported sleep measures, including sleep duration, daytime sleepiness, and insomnia.
3. To examine the effect of vitamin D supplementation without additional calcium supplements on BMD.

3 Materials and Methods

3.1 Study design and methods

3.1.1 Study populations and recruitment

Paper I

The Tromsø Study, constituting the fundament of Paper I, is a longitudinal population-based health study conducted in the municipality of Tromsø, Northern Norway. The main intentions and design of the study has been described in detail before (159). In short, seven repeated surveys have been conducted between 1974 and 2016 (Tromsø1–Tromsø7), to which total birth cohorts and random samples have been invited to participate. Geographically situated at 69° North, Tromsø has extreme year-around variations in daylight and sun exposure, with two months of polar night during winter and two months of midnight sun during summer. Thus, the Tromsø Study provides an exceptional environment to study the relationship between vitamin D and sleep. The data for Paper I was collected in Tromsø7 (2015-16), to which all Tromsø inhabitants aged 40 years and older (N=32,591) were invited by mail to participate. A total of 21,083 men and women between the age of 40-99 years accepted the invitation (attendance 65%). Along with the invitation to participate, each participant received a four pages questionnaire (Q1, Appendix 1) for assessment of general characteristics (as described in detail in section 3.2) to be completed on paper or online. A second, more extensive questionnaire (Q2, Appendix 2) was also provided, including questions regarding habitual sleep (as described in detail in section 3.2). This second questionnaire was completed online either prior to or when attending the study visit, during which the participants also underwent clinical examinations (e.g., height and weight), and biological sampling (e.g., serum samples for s-25(OH)D assessment).

Paper II

The double-blind RCT "The Effect of Vitamin D Supplementation on Cardiovascular Risk Factors" (D-COR) constitute the fundament of Paper II and has been described in detail before (160). In short, the D-COR study was a 4-month placebo-controlled vitamin D intervention study performed between June 2015 and December 2016. The primary aim was to evaluate the effect of vitamin D supplementation on cardiovascular risk factors in participants with low levels of s-25(OH)D. Based on the ongoing work with Paper I, a sleep

questionnaire was added about half-way into the main study to also investigate the effect of vitamin D supplementation on self-reported sleep quality. In total, 1,489 participants were invited by mail after having participated in Tromsø7 (described above). Both men and women below 80 years of age with s-25(OH)D values below the 10th percentile in Tromsø7 (< 42 nmol/L) were invited, of which 639 responded to the invitation. All the participants were screened for eligibility prior to participation, and the screening was performed via telephone interviews by study nurses at the Clinical Research Unit at the University Hospital of North Norway (UNN). Of the 455 participants who passed the initial telephone screening and met at the baseline visit, 33 participants did not meet the inclusion criteria. Participants were excluded if having known granulomatous disease, diabetes, history of renal stones last five years or serious disease that would complicate participation in the study. Participants were also ineligible if using vitamin D supplements exceeding 800 IU vitamin D per day, solarium on a regular basis, and/or planning a sunny holiday(s) during the study period. Women of childbearing potential (below 50 years of age) without use of acceptable contraception (hormonal, IUD) were also excluded. In addition, as the aim of Paper II was to evaluate the effect of vitamin D supplementation on self-reported sleep measures, participants who were missing s-25(OH)D measurements and/or baseline or end-of-study values for all sleep measures were excluded from the analyses (n=645).

Paper III

The double-blind RCT "Prevention of Diabetes Type 2 with Vitamin D Supplementation in Subjects with Reduced Glucose Tolerance" constitute the fundament of Paper III and has been described in detail before (161;162). In short, the study was a 5-year placebo-controlled vitamin D intervention trial conducted from March 2008 till May 2015. The primary aim was to evaluate vitamin D for the prevention of type 2 diabetes mellitus. As the effect of vitamin D supplementation on BMD without additional calcium has not been settled, measurement of BMD was also performed to investigate this as a secondary outcome. Most participants were recruited after having participated in the sixth survey of the Tromsø Study (Tromsø6, 2007-2008) (163). A few participants were also recruited via the out-patient clinic at UNN (164;165). In total, 511 men and women between the age of 38-80 years and diagnosed with prediabetes[1] were randomized at baseline. Exclusion criteria were primary

[1] Impaired fasting glucose (IFG) 6.0–6.9 mmol/L and/or impaired glucose tolerance (IGT) with fasting serum glucose < 7.0 mmol/L and 2-h value 7.8–11.0 mmol/L at oral glucose tolerance test (OGTT) with 75 g glucose.

hyperparathyroidism, sarcoidosis or other granulomatous disease, history of urolithiasis, cancer diagnosed in the last five years, unstable angina pectoris, acute myocardial infarction, or stroke in the last year, or reduced kidney function (creatinine above 125 μmol/L in men and 105 μmol/L in women). Participants with allergies to nuts (as the placebo capsules contained arachis oil from peanuts) and women being pregnant, lactating or of fertile age (< 50 years) without use of acceptable contraception (hormonal, IUD) were excluded. In addition, as the aim of Paper III was to examine the effect of vitamin D supplementation on BMD, participants missing s-25(OH)D and/or BMD measurements, or reporting to use bisphosphonates, were excluded from the analyses (n=97).

3.1.2 Study medication and compliance

In both RCTs (Paper II and III), the study medication consisted of capsules with vitamin D3 (Dekristol cholecalciferol capsules, 20,000 IU (500 μg)) or identical-looking placebo capsules (containing arachis oil). In Paper II, the participants were instructed to take five capsules as a loading dose (100,000 IU) followed by one capsule weekly (20,000 IU/week). In Paper III, the weekly dosage was identical to that in Paper II, but no loading dose was given.

In Paper II, compliance was calculated as the ratio of capsules used (capsules supplied minus capsules returned) to number of weeks between V2 and V4. In Paper III, compliance was calculated as the ratio between capsules used and capsules supplied for that time period.

3.1.3 Randomization and blinding

In both intervention studies (Paper II and III), randomization to the vitamin D and placebo group was performed by the central randomization unit at the Clinical Research Centre at UNN, using a block randomization procedure. In Paper II, the randomization was stratified according to sex, vitamin D status in Tromsø7 (above/below 25 nmol/L), smoking status and body mass index (BMI) (above/below 27 kg/m^2, self-reported height and weight). In Paper III, the randomization was performed non-stratified. In both studies, the randomization number was sent to the Clinical Research Unit for registration and to the hospital's pharmacy for distribution of the appropriate study medication. Except for the pharmacy, having no contact with the study participants, all the study personnel (nurses, doctors, and main investigators) were blinded to the randomization throughout both studies.

3.1.4 Ethics and safety (Paper I-III)

All the participants provided informed, written consent prior to the examinations to participate in the studies. The studies were approved by the Regional Committee for Medical Research Ethics (REK Nord), identifications numbers being 81/2007, 2014/940 and 2013/1464 for the Tromsø Diabetes Study, Tromsø7 and the D-COR Study, respectively. In addition, the Tromsø Diabetes Study and the D-COR study were approved by the Norwegian Medicines Agency (identifications numbers being 2007-002167-27 and 2013-003514-40, respectively) and registered at ClinicalTrial.gov (identifications numbers being NCT00685594 and NCT02750293, respectively). All research data was handled strictly confidential, and the data sets were made available without names or personal identification numbers in all three studies. In both RCTs, the participants were informed about the risk and symptoms of hypercalcemia and instructed to contact the Clinical Research Unit at UNN or one of the investigators if developing such symptoms. Information was also given regarding insurance, in which the participants were covered by the Norwegian patient injury compensation, as well as by a separate liability insurance connected to clinical drug trials. Adverse events were specifically asked for at all visits during the trial period and were monitored as described in the respective papers (Paper II and III).

In all three studies, the participants were informed that the results of all blood tests and other measurements would be available as part of their hospital record and stored in the hospital's computer system, and that recommendations of treatment and follow-up would be provided after participation in the study was ended.

In Paper II, participants with insufficient levels of vitamin D, based on both Norwegian and international standards, were included. This raises an important ethical question regarding whether it was right to use placebo, instead of a low dose of vitamin D. The decision to use placebo was deemed ethically defendable, considering that the recommended vitamin D level is based on cross-sectional studies only and that strong evidence of health benefits from supplementation with vitamin D (except for those regarding the skeleton) is currently lacking. Nevertheless, to minimize the risk associated with participation in the study the intervention period was ended after 4 months and, at the end of the study, all participants (regardless of treatment allocation) were advised to use vitamin D supplementation 800 IU with a scheduled control after one year (160).

Finally, the participants were provided a gift card to cover travel expenses in both RCTs (Paper II and III).

3.2 Measurements

3.2.1 Assessment of general characteristics

In Paper I, general characteristics were assessed using self-administered questionnaires. First, the participants filled in a general questionnaire (Q1, Appendix 1) collecting information about overall health status and modulating risk factors, including socio-economic relations, physical activity, diet, alcohol intake, smoking status, previous medical history and use of medications. Second, a more extensive questionnaire (Q2, Appendix 2) was filled in, including questions regarding habitual sleep (as described in detail below), vitamin D supplement use, use of sunbeds and recent sunny holiday(s). The questionnaires were filled in online either prior to or during the study visit, and technical assistance was provided by trained personnel at the study site if needed.

In both RCTs (Paper II-III), the participants were interviewed by trained personnel at baseline to have their medical history taken, including questions on smoking status, medical history, medications, and dietary supplements. This information was collected again at the end of the study in Paper II, and during each annual visit throughout the study in Paper III.

In Paper II, a self-administered questionnaire was filled in at baseline and the end of the study to collect information regarding habitual sleep (as described in detail below).

3.2.2 Clinical examinations and sample taking

Height and weight were measured with the participants wearing light clothing and without shoes. BMI was calculated as weight (kilograms) divided by height squared (meter^2).

In Paper I, serum samples for population characteristics were performed non-fasting. In Paper II serum samples (both fasting and non-fasting) were drawn at baseline and at the end of the study. In Paper III, serum samples (both fasting and non-fasting) were drawn at baseline, annual visits, and every six months in between annual visits (the latter for safety monitoring purposes). All measurements were performed by trained technicians in all three studies. Serum samples were stored at -70 degrees Celsius and analysed consecutively within 24 hours (Paper I), or after the study was completed (Paper II and III).

3.2.3 Laboratory analyses and 25-hydroxyvitamin D measurements

In all three studies, measurements of s-25(OH)D were analyzed at the Department of Clinical Biochemistry at UNN. Both s-25(OH)D_3 and s-25(OH)D_2 (the sum of which is presented as s-25(OH)D) were detected using an in-house liquid chromatography with a tandem mass spectromy (LC-MS/MS) method. The analysis had a CV < 9% for both assays and no known interference from other substances. The LC-MS/MS-analysis at UNN is accredited by the Norwegian Accreditation Authority, and the laboratory participates in the international quality surveillance program DEQAS to ensure the analytical reliability of the 25(OH)D assays. In accordance with recent recommendations (31, 32), subgroup classification of vitamin D status was based on s-25(OH)D level; Vitamin D deficiency was defined as s-25(OH)D values < 30 nmol/L, insufficiency as values from 30 nmol/L to 50 nmol/L, sufficiency as values from 50 through 75 nmol/L, and values > 75 nmol/L were categorized as high.

All other laboratory analyses (including calcium and PTH) were performed as described in the respective papers.

3.2.4 Self-reported sleep measures (Paper I and II)

Sleep duration

The participants were asked to select from pre-specified alternatives which best represented their habitual bedtime, rise time and SOL (i.e., the average minutes from bedtime to falling asleep). Values were reported separately for weekends and weekdays, in which the weekday values were used for subsequent analyses. Sleep duration was calculated by subtracting SOL from time in bed (bedtime subtracted from rise time). Sleep duration was dichotomized into normal (7-9 hours) and inadequate sleep duration (ISD) (< 7 or ≥ 9 hours), in accordance with the National Sleep Foundation's recommendation for adults (50).

Insomnia

To assess symptoms of insomnia, a modified version of the Bergen Insomnia Scale (BIS) was used (166). The modifications were made to adhere with recent guidelines on diagnosing insomnia, including the International Classification of Sleep Disorders, Third Edition (ICSD-3) (71) and the Diagnostic and Statistical Manual for Mental Disorders, Fifth Edition (DSM-5) (72), and has been described in detail before (63). In short, the original BIS assessed sleep patterns during the last month and included a question regarding non-restorative sleep. According to the DSM-5 and ICSD-3, the minimum symptom duration for insomnia is set to

three months and non-restorative sleep is no longer an obligate criterion for the diagnosis. **Table 1**. summarizes the three criteria that were applied to differentiate between participants with and without insomnia.

A.	At least one of the following nocturnal symptoms: 1) prolonged SOL 2) difficulties maintaining sleep 3) and/or early morning awakening	≥ 3 nights/week
And:		
B.	One or both of the following daytime symptoms: 1) daytime sleepiness/tiredness 2) dissatisfaction with sleep	≥ 3 days/week
And:		
C.	Duration of sleep problems	≥ 3 months

Table 1. The three criteria used to differentiate between participants with and without insomnia.

Excessive Daytime Sleepiness

EDS was assessed using the Epworth Sleepiness Scale (ESS) (167). The ESS is a 4-point Likert-scale questionnaire composed of 8 items, in which the participant marks the probability of dozing in routine situations, such as watching television, lying down to rest, and being a passenger in a vehicle. The score for each item varies from 0 (no chance of dozing) to 3 (high chance of dozing). The total score ranges from 0-24 points and a score ≤ 10 points indicates normal daytime sleepiness, whereas higher scores indicate EDS. Further, EDS may be categorized as mild (11-12 points), moderate (13-15 points) and severe (16-24 points). Validation studies of the ESS has been carried out in several languages, including Norwegian, with acceptable internal consistency and test-retest reliability (168).

3.2.5 Bone mineral density (Paper III)

In Paper III, BMD was measured at baseline and at the end of the study. The measurements were obtained at the femoral neck and total hip measurement sites using DXA (GE Lunar Prodigy, Lunar Corporation, Madison, WI, USA). Calibration of the scanner was performed daily against the standard calibration block provided by the manufacturer, and the measurements showed no drift throughout the study. BMD measurements were classified as normal if corresponding to a T-score ≥ -1.0 standard deviations of the mean of healthy young women. T-scores < -1.0 were classified as osteopenic, since no men, and only very few women, presented with osteoporotic T-scores (≤ -2.5).

3.3 Statistics

In all three papers the statistical tests were performed two-sided and *P*-values < 0.05 were considered statistically significant. Normal distribution was evaluated by visual inspection of histograms and by determination of kurtosis and skewness. Normally distributed data was presented as means and standard deviations and non-normally distributed data as medians and ranges (minimum-maximum). Proportions were provided as numbers and percentages in all three papers, except in Paper III, in which only percentages were provided.

In Paper I, one-way analysis of variance (ANOVA) was used to explore differences in the continuous sleep measures (i.e., sleep duration and ESS-score) across s-25(OH)D groups (as previously described). The prevalence of the categorical sleep measures (i.e., ISD, insomnia, and EDS) in Paper I was compared across s-25(OH)D groups using the Pearson's χ-square test. This was also applied in Paper II-III to compare differences between the vitamin D and placebo group in the categorical variables at baseline. In Paper II, changes from baseline to the end of the study in the categorical sleep measures were compared between the vitamin D and placebo group using the Fisher's exact test (as some variables showed cell counts less than 5). Differences between baseline and end-of-study s-25(OH)D were compared within the vitamin D and placebo group using a paired-sample t-test (Paper II-III). In all three papers, between-group differences in normally distributed, continuous variables (e.g., s-25(OH)D, sleep duration, ESS-score, and delta BMD) were tested using a Student's *t*-test when comparing two independent groups (e.g., within each categorical sleep measure in Paper I, and between the intervention groups in Paper II-III). In Paper III, differences between the vitamin D and placebo group regarding non-normally distributed variables (that could not be

normalized with log-transformation) were tested using the non-parametric Mann-Whitney U-test.

In Paper I, the risk of having each of the three categorical sleep measures (i.e., ISD, insomnia, and EDS) were estimated and compared across s-25(OH)D groups using multivariate logistic regression. To guide the analyses, Directed Acyclic Graphs (DAGs) were constructed to identify the minimal sufficient adjustment sets (i.e., the lowest number of covariates required to control for confounding). Models were then fitted to the data and odds ratios (ORs) with corresponding 95% confidence intervals (CIs) were calculated.

Multiple linear regression analyses were performed in all three papers. In Paper I, the association between s-25(OH)D and the continuous sleep measures (i.e., sleep duration and ESS-score) was assessed using the same adjustment sets as in the logistic regression analyses. In Paper II, end-of-study values of the continuous sleep measures (i.e., sleep duration and ESS-score) were compared between the intervention groups, both stratified by and adjusted for sex, in addition to adjustment for age and baseline values of the sleep measure. In Paper III, multiple linear regression was used to evaluate predictors of baseline BMD. If the change in BMD (delta values calculated as end-of-study value minus baseline value) differed significantly between the intervention groups, this was further tested with linear regression, adjusting for baseline values, observation time and variables significantly predicting BMD at baseline (as described in detail in Paper III).

Potential interactions between relevant variables were tested by adding one interaction term at a time into the regression analyses, assessing the corresponding *P*-value. In Paper I, there were no statistically significant interactions between s-25(OH)D and age, sex, BMI, season, smoking status, or symptoms of psychological distress, except in the analyses of insomnia, in which a significant interaction was noted between s-25(OH)D and sex (p =.012). In Paper II-III, there were no statistically significant interactions between allocation status (vitamin D versus placebo) and age, sex, BMI, smoking status, or symptoms of psychological distress. However, since sex-differences have been reported in previous literature regarding both sleep and BMD, the results were presented sex-stratified in all three papers.

All statistical analyses were performed using IBM SPSS software version 24-27 (IBM Inc., Chicago, Illinois). In addition, the DAGitty v3.0 software was used to construct the DAGs in Paper I.

4 Summary of results

Paper I

The aim of Paper I was to examine the association between s-25(OH)D and self-reported sleep measures in a large cross-sectional study population (Tromsø7, 2015-16). A total of 20,438 individuals were included in the analyses after having excluded participants with missing s-25(OH)D measurement and/or no data on any sleep measures (n=645). Adjusted for month of serum sampling, there was a positive association between s-25(OH)D and sleep duration, which was reflected in the lower odds of ISD with higher s-25(OH)D in both men and women. All estimates were attenuated with additional adjustment for relevant confounding factors, such as age, BMI, smoking, alcohol intake, leisure time physical activity, marital status, self-perceived economy, shift work, OSA, psychological distress and/or presence of other relevant comorbidities (including cardiovascular disease, cancer and/or chronic pain), as well as afflictions of night sweats (the latter in women only). With full adjustment, statistical significance for the association between s-25(OH)D and sleep duration and ISD depended on sex and whether the analysis was modelled using s-25(OH)D as a continuous or categorical variable in the analyses. Modelled using linear regression, sleep duration was positively associated with the continuous s-25(OH)D variable, but the association was only significant in women. When using the categorical variable, the s-25(OH)D insufficient group had significantly shorter sleep duration compared to the sufficient group in both sexes. Modelled using logistic regression, higher levels of s-25(OH)D (continuous) was inversely associated with lower odds of ISD, but the association was only statistically significant in men with full adjustment. When using the categorical s-25(OH)D variable, the association remained statistically significant only in women with s-25(OH)D insufficiency compared to women with sufficient levels. Regarding insomnia, there were no statistically significant associations with s-25(OH)D in men. In women, the odds of insomnia was significantly higher in women with high s-25(OH)D compared to women with sufficient levels, which persisted with full adjustment for confounding factors. There were no associations between s-25(OH)D and daytime sleepiness or EDS in the fully adjusted models, except significantly lower ESS-scores among women with high s-25(OH)D compared to women with sufficient levels. In summary, a tenuous association was found between s-25(OH)D and self-reported sleep measures, but the results conflicted according to sex, method of analysis and the sleep measure studied. Given the cross-sectional design of the study, causal inferences could not be drawn.

Paper II

The aim of Paper II was to evaluate the effect of vitamin D supplementation on self-reported sleep measures. In total, 189 participants (92 given vitamin D and 97 given placebo) were included in the analyses, having completed measurement of s-25(OH)D and completed the study sleep questionnaire at baseline and at the end of the study. After four months, vitamin D supplementation compared to placebo had no statistically significant effects on neither sleep duration, nor ESS-score, and there were no statistically significant differences according to allocation status regarding the incidence or recovery of ISD, EDS or insomnia, neither in men, nor in women. Post-hoc analyses regarding the effect of vitamin D supplementation on sleep duration and ESS-score showed no statistically significant differences between the intervention groups according to baseline or end-of-study s-25(OH)D, nor according to sleep status at baseline. In summary, four months supplementation with vitamin D versus placebo in participants with low s-25(OH)D did not have statistically significant effects on self-reported sleep measures.

Paper III

The aim of Paper III was to evaluate whether vitamin D supplementation without additional calcium could have a positive effect on BMD (i.e., attenuate bone loss and/or increase BMD) In total, 414 participants (201 given vitamin D and 213 given placebo) were included in the analyses, having completed measurements of s-25(OH)D and BMD at baseline and at the final visit. After a median observation time of 59 months, men given vitamin D had significantly less reduction in BMD at the femoral neck measurement site compared to men given placebo (0.000 versus − 0.010 g/cm2, $p = 0.008$). No statistically significant differences between intervention groups were seen at the total hip measurement site, neither in men, nor in women. In summary, supplementation with vitamin D without additional calcium was demonstrated to attenuate bone loss in men with prediabetes.

5 Discussion

5.1 Methodological considerations

The fundament of this thesis is based on data from one cross-sectional population-based study and two intervention studies (RCTs). In the following, each study type will be discussed with regards to their strengths and limitations, and how the internal and external validity of our findings may have been affected by systematic and random errors.

5.1.1 Study design

The first paper in this thesis was based on data from the Tromsø Study, a community-based health study conducted in Northern Norway. As is typical for population-based studies, the Tromsø Study is frequently used to explore associations between environmental exposures and diseases. The analyses of Paper I included more than 20,000 individuals. Large population samples enables the researcher to identify small associations that are otherwise difficult to demonstrate, although simultaneously heightening the risk of discovering statistically significant associations of little to no clinical relevance (169). It cannot be excluded that this occurred in Paper I, especially when considering the moderate ORs. Thus, the associations found between s-25(OH)D and self-reported sleep measures may be of less relevance in the clinical setting compared to other adjusted risk factors, such as shift work, OSA, depression, chronic pain and/or poor general health status. Moreover, observational studies on vitamin D are prone to bias resulting from reversed causality; People of good health, getting sufficient amounts of sleep, are more likely to spend time outdoor (getting exposed to sunlight), being physically active and making healthy choices when it comes to their diet – all promoting vitamin D status. Dietary and solar sources of vitamin D also contain other mediators promoting health and well-being, which implies that many of the health benefits attributed to vitamin D may represent co-variation, rather than causation. As cross-sectional studies measures the exposure and outcome variables at the same time, without information about which came first, conclusions about cause/effect cannot be drawn from this type of study (170).

It has been argued that *"[t]he only way to prove a causal role of vitamin D in health is through properly designed RCTs"* (171). By randomly allocating participants to an intervention, the RCT study design inherently balance potential confounders (both measured and unmeasured) between the intervention groups. This way, differences in the outcome can be attributed to the intervention alone, without concerns about confounding. RCTs are

considered the gold standard when assessing therapeutic interventions but are not without potential limitations or biases (172). In general, RCTs are expensive to perform and time-consuming to both researchers and participants. The higher burden associated with participation typically decreases the respondent's willingness to participate. Combined with strict inclusion/exclusion criteria, this negatively impacts the response rate (as discussed in further detail regarding external validity). It is often challenging to reproduce the results of RCTs in a less controlled environment such as the clinical setting. In particular, long-term outcomes that requires participation over many years, such as in Paper III, may reduce compliance and adherence to study protocols. However, these factors were both exceptionally high in Paper III.

In addition, limitations specific to nutrient supplementation trials, including RCTs with vitamin D, are important to consider. Easy access to out-of-study supplements and laboratory measurements of s-25(OH)D in the general practitioner service, increases the risk of contamination and unblinding (as discussed in further detail regarding internal validity). The association between vitamin D and several health outcomes follows a sigmoid pattern (as for nutrients in general), which implies that additional supplementation is not be expected to reduce the risk of developing the outcome if given to individuals that are vitamin D sufficient to start with (172;173). Thus, recruitment of vitamin D sufficient people to RCTs with vitamin D decreases the power of the study to detect beneficial effects; As rhetorically asked by Robert Scragg "*Would we include thin people in a trial of obesity reduction?*" (172). This is especially relevant for Paper III, in which the mean s-25(OH)D level was relatively high in the study population at baseline.

The power of RCTs to demonstrate effects of an intervention also depends on the size of the effect, and the sample included to detect it; The smaller the effect, the larger the sample has to be to provide precise effect estimates. Paper II-III were both secondary analyses of previously performed trials, with sample sizes calculated based on the primary outcomes. Considering the width of the 95% CIs (especially in Paper II), the estimates ought to be interpreted conservatively (174). As neither of the RCTs analysed in Paper II-III were designed to test the effect of vitamin D supplementation on self-reported sleep measures (Paper II) or BMD (Paper III), this must be considered as an evident short-coming of the present thesis.

5.1.2 Internal validity

For the findings of a study to be true for the population investigated, high internal validity is an important premise. There are three main types of systematic errors (also known as biases) that may compromise the internal validity; confounding, selection bias, and information bias.

Confounding

Systematic differences between variables that are not causally related, but rather induced by the presence of a third variable (measured or unmeasured), is known as confounding (175). To be considered as a potential confounder, three criteria must be satisfied (175). First, the variable must be associated with the outcome, in that the risk of the outcome is either increased or decreased in its presence (or absence). Second, the variable must be associated with the exposure, in that its distribution must be unequal across the exposure groups. Third, the exposure causing the outcome may not also cause the confounding variable. Confounding is particularly prevalent in observational studies, such as the Tromsø Study, which Paper I is based on. At worse, bias caused by confounding may alter the precision, and even the direction, of the estimates.

Appropriate control for confounding factors, is crucial in observational data analysis, especially in outcomes with multifactorial causes, such as sleep disturbances. In general, various measures may be used to counteract confounding in research, including stratification, regression adjustment, restriction, matching, and randomization. However, inappropriate use of these measures, may introduce overcontrol bias and/or endogenous selection bias (176). To control for confounding and to avoid introducing unintended bias, DAGs were constructed to guide the statistical analyses in Paper I. With a combination of mathematical rules and simple drawings, DAGs (illustrated in Figure 3) may be used to guide the statistical analyses to obtain unbiased estimates. These causal diagrams emphasizes the use of expert knowledge when evaluating relationships between variables and may be used to visualize confounding and selection bias (177).

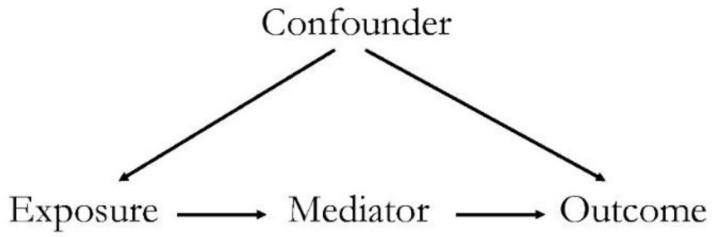


Figure 3: The basic structure of a directed acyclic graph.

With stratification, distinct groups or layers of data may be analysed separately to display differences between subgroups, or strata, within the study sample. In the present thesis, stratification was done according to sex in all three papers. Given that sex differences in both sleep and BMD are well-documented and biologically plausible, relevant differences might have been missed if the data in men and women had been pooled.

Given the age-span of 40-99 years in Paper I, postmenopausal women presumably made up the majority of women in the study sample. Considering that hormonal changes during menopause increases the risk of sleep disturbances (67) and that s-25(OH)D have been inversely associated with female sex hormones, including oestradiol (178), menopausal status was considered a potential confounder on the association between s-25(OH)D and sleep in women, in particular in the presence of night sweats. In Paper I, the ORs in women were adjusted for afflictions of night sweats, serving as a proxy of menopausal status. However, this additional adjustment had moderate to no influence on the estimates and seemed to be less important than anticipated.

In Paper I, factors increasing s-25(OH)D (i.e., using a vitamin D supplement, taking solarium and/or having been on a recent sunny holiday) were not adjusted for, as the aim was to estimate the association between self-reported sleep measures and s-25(OH)D from all sources. Women with high levels of s-25(OH)D may still have differed from those with lower levels in other (unmeasured) ways with the potential to influence self-reported sleep measures, such as having a more health-conscious personality. Also, if women with insomnia were more likely to start using a vitamin D supplement in an attempt to ameliorate symptoms, then the high vitamin D status would be a result, rather than the cause, of insomnia. The reader is referred to section 5.2.1 for a detailed discussion regarding the risk of reversed causation in Paper I.

In general, if distortion of study results persist after controlling for a confounding variable this is known as residual confounding (175). Residual confounding is typical when continuous or discrete variables are converted to categorical variables, as some information is typically lost upon categorization. However, given the categorical nature of the clinical every-day practice, using categories may also provide valuable information about non-linear associations, which may be overlooked using continuous variables only. In Paper I, the majority of the applied covariates were included as categorical variables only (e.g., physical activity, smoking status, presence of chronic pain), as appropriate alternatives (e.g., total energy expenditure, pack-

years, and pain score) were unavailable. The sleep measures and s-25(OH)D exposure variables in Paper I were analysed using both the continuous variables (i.e., sleep duration and s-25(OH)D value) and the categorical variables (i.e., ISD and s-25(OH)D categories). There was no tendency of statistically significant results only when the continuous variables were used.

The study design of Paper II-III (i.e., RCTs) inherently prevents confounding at baseline by allocating the participants to an intervention at random. Although confounding at baseline was unlikely to have influenced the results of Paper II-III, bias may have emerged during follow-up. Post-randomization confounding may introduce bias in estimates of treatment efficacy, as well as altering the power of a study to detect a true treatment effect due to incomplete or differential adherence to the protocol or to use of rescue medications during the study (179;180). This is a common challenge in intervention trials with vitamin D (and other nutrients), given the widespread availability of high dose, over-the-counter supplements, and laboratory measurements of s-25(OH)D (and other nutritional markers) in the general practitioner service (181). However, both RCTs included in this thesis were well-conducted and reported excellent adherence to the trial regimen, as described in the respective papers. Also, when comparing s-25(OH)D measurements between the vitamin D and the placebo groups at baseline and at the end of the study, the placebo group remained unchanged in both studies.

In Paper II, the RCT was originally designed to evaluate the effect of vitamin D on cardiovascular risk factors. Thus, information regarding other types of sleep treatments (e.g., cognitive behavioural therapy) was not collected. If treatment with vitamin D had been effective, the participants in the placebo group might have been more likely to initiate such treatments, which could have biased the results towards the null. Nevertheless, restricting the analyses to the participants not reporting to use sleep medications (or anti-depressants), did not change the results (unpublished findings).

Selection bias

Selection bias refers to the systematic inclusion of individuals, groups, or data, to a study or analysis that differ consistently from the population of interest (182). This may either induce or dilute an association between the exposure and the outcome. In Paper I we used data from the population based Tromsø Study. Findings from another population-based health study in Norway, The Trøndelag Health Study, have shown that individuals who participate in

population surveys have lower mortality rates, smoke less and have better general health than those who do not (183), also known as the "healthy volunteer effect" (184). It cannot be excluded that the prevalence of vitamin D deficiency and sleep disturbances may have been higher among non-attendees compared to those who agreed to participate. Thus, non-attendance in the Tromsø Study may have introduced selection bias in Paper I. To what degree this may have influenced the results is uncertain, but a dilution of the association would be more likely, than to have entailed a non-existing relationship.

Selection bias is of less concern with the RCTs study design, which Paper II-III were based on. Nevertheless, because the participants in these trials were invited based on their previous participation in the Tromsø Study, selection bias related to the "healthy volunteer effect" as discussed above, must be considered when interpreting the results of these studies. The participants in Paper II were informed of their vitamin D status through the invitation to participate in the study. Given the considerable attention devoted to extra-skeletal effects of vitamin D in mainstream media over the last years, this could potentially have increased motivation to participate in the study, as well as the probability of reporting sleep complaints on study visits, potentially causing falsely high prevalence estimates. However, compared to previous reports in the Tromsø Study, the observed prevalence of the included sleep measures was comparable to those previously reported (63;185).

An important premise for the assertion that selection bias is avoided when using an RCT study design, is that the randomization process remains unbiased by the researchers' or the participants' knowledge of the given treatment prior to and during the study. This is particularly relevant with regards to Paper II, in which the outcome was subjectively assessed. The study was performed double-blind, meaning that neither the researchers, nor the participants, had information on the randomization code. Thus, the information provided by the participants and the researchers' conclusions regarding benefit of supplementation were unlikely to have been influenced by this kind of bias. Nevertheless, imbalances can and do occur, even in adequately blinded RCTs, especially with smaller study samples (186). Imbalances may be accounted for by using stratified randomization, as was done in Paper II (as described in detail in the methods section). This way, the researcher may ensure that roughly equal numbers of participants with certain characteristic are allocated to each treatment group. Stratified randomization was not performed in Paper III. However, baseline characteristics were compared between the intervention groups prior to the main analyses in Paper III to establish that the groups did in fact have similar characteristics.

Missing data represents another challenge in observational, as well as interventional studies. In general, the more information that is missing from an analysis, the more cautious should one be when interpreting the results of a study. In the presence of missing data, the true association or treatment effect may not be reflected by the study sample. The sleep questionnaire in Paper II was included half-way into the main study, and so only half the sample (n=189) was available for the analyses. Nevertheless, it is unlikely that this would have introduced selection bias, as the randomization was performed continuously throughout the trial execution period. Also, the participants from the main study who were missing information on sleep measures, and therefore not included in the study (n=222), did not differ significantly from the included with regards to age, sex, BMI, or smoking status at baseline. The excluded participants had significantly lower mean s-25(OH)D compared to those included. The reason for this may have been that most of the participants that were included before the sleep questionnaire was implemented were invited to participate if having s-25(OH)D below 30 nmol/L in Tromsø7. This was later expanded to include all participants with s-25(OH)D below the 10^{th} percentile in Tromsø7 (< 42 nmol/L) to reach the estimated sample size. This may potentially have contributed to a dilution effect on the association between s-25(OH)D and self-reported sleep measures, given that there was one.

Information bias

Another source of systematic error, information bias, occurs when inaccuracy in measurement or estimation of data leads to misclassification of participants in dichotomous variables with regard to the exposure or outcome variables. This may over- or underestimate the findings of a study, or even bias the results towards the null (i.e., no association/effect), depending on whether the misclassification is differential (non-random) or non-differential (random). The first type, differential misclassification, occurs when this is dependent on the outcome or exposure variable (i.e., being differential across groups) (182). The latter, non-differential misclassification, occurs randomly across the exposure and outcome group (182). In Papers I-II, information about the outcome (self-reported sleep measures) and potential confounders (lifestyle habits, socioeconomic factors, and chronic diseases) was collected using self-administered questionnaires. These were filled in by the participants during or prior to the study visits. Self-report may increase the risk of misclassification, especially regarding information about non-healthy lifestyle habits, which is often underreported (187;188). As the participants in Tromsø7 were not aware of the association being investigated in Paper I, it is unlikely that this type of underreporting would have differed between the exposure groups

(i.e., across vitamin D categories) or depending on whether or not the participants reported one of the relevant sleep measures (i.e., ISD, EDS or symptoms of insomnia). Thus, information bias regarding lifestyle habits would likely have been non-differential, potentially diluting the association between s-25(OH)D and self-reported sleep measures.

The questionnaire items used to calculate sleep duration in Paper II, included imprecise assessments of bedtime (i.e., "After 2 am" / "Before 8 pm"), rise time (i.e., "Before 5 am" / "After 11 am") and SOL (i.e., "30-45 minutes", "45-60 minutes" and ">60 minutes"). As a consequence, the estimated sleep duration in 36 of the participants was associated with more or less degrees of uncertainty. A total of seven participants reported a sleep duration that could be categorized both as ISD and as normal. For instance, if a participant reported to go to bed at 00:30 and woke up at 08:00, but used 30-45 minutes from bedtime to fall asleep, this would translate into a sleep duration of at least 6 hours and 45 minutes (i.e., categorized as ISD), but possibly as much as 7 hours (i.e., categorized as normal). However, information bias resulting from this type of misclassification would likely have been non-differential as the categorization of a participant as having or not having the outcome (i.e., ISD) would be independent of the exposure (i.e., treatment allocation). Excluding these participants from the linear (*n* excluded = 36) and logistic (*n* excluded =7) regression analyses did not change the results (unpublished findings).

5.1.3 External validity

An overall goal in all research is to ensure that the results are valid and useful, at least at some level, for a broader group of people or situations after the study has ended. Thus, conscious selection of the study population is highly relevant both to ensure generalizability of the results, and with regards to internal validity (as discussed above).

In all three papers, the participants were primarily recruited after having participated in Tromsø6 or Tromsø7 (as described in the methods section). These cohorts mainly consisted of Tromsø inhabitants aged ~40 years or older, most of whom were of Caucasian origin. In addition, the very old adults (i.e., those aged 80 years and older) were excluded in both Paper II-III. Thus, the results of these papers ought not to be generalized to persons outside this age group or to persons of non-Caucasian ethnicity.

The studies in Paper II-III were both RCTs with strict eligibility criteria (as described in detail in the methods section). Extensive inclusion and exclusion criteria are common traits of

intervention studies, that are necessary to ensure security, eliminate potential confounders and ease the interpretation of results, especially when the sample size is small to moderate. However, excluding large groups of individuals has consequences for the generalizability of the results, which may only be considered valid when applied in an equally selected population.

Both in observational studies (such as in Paper I) and in interventional studies (Paper II-III), the respondents who agree to participate are usually healthier than the population average. The consequence of studying such a selected group of the so-called "worried well" is poor external validity and uncertainty as to whether the results are valid when applied in the general population (172;183;184).

5.1.4 Random error

Science is about forming hypotheses to be tested against evidence based on real-world data. The goal is to enable the researcher to make valid conclusions about the hypotheses formed, rejecting, or accepting their premises. However, changes in research conditions that are unpredictable or unknown may decrease the precision of estimates, potentially distorting the results of a study. Such random errors may occur as a consequence of imprecision in measurement, data processing, calculation, or analyses. Although not possible to avoid completely, random errors may be averted by optimizing study design and sample size. Application of appropriate statistical methods can further minimize their impact on the results. All three papers included in this thesis applied a predefined α of 0.05, meaning that the null hypothesis was rejected for all p-values < 0.05. As formulated by Andrew Vickers «*The p-value is the probability that the data would be at least as extreme as those observed, if the null hypothesis were true*» (189). Following the normal distribution, a statistically significant association or treatment effect (i.e., rejecting the null hypothesis) would be expected to occur simply by chance once with every 20 hypotheses tested, using 0.05 as the level of significance. This is known as the type I error rate and is the probability of rejecting the null hypothesis when it is true (i.e., a false positive finding). In Paper III, we found a statistically significant positive effect on BMD, but only at one of the two measurement sites and only in men. Also, no effect was found in subgroups based on s-25(OH)D at the end of the study, nor according to baseline s-25(OH)D. The risk of reporting false positive findings increases with each test performed, and some heterogeneity in treatment effect is expected across subgroups simply by chance. Thus, it cannot be excluded that the positive effect on

BMD in men represents a type I error. Type I errors in multiple testing may be addressed using Bonferroni correction, in which the predefined alpha level is divided by the number of tests performed, or alternatively by multiplying each p-value by the same number (190). Neither of the included papers in this thesis applied Bonferroni corrections. The method is considered conservative, as the risk of overadjustment is high, especially if the different endpoints are correlated (as was the case between the BMD measurement sites in Paper III).

If the number of participants included in a study or subgroup is too small (or the drop-out rate is higher than expected), the risk of falsely accepting the null hypothesis increases (191). This is known as a type II error and may be prevented by performing adequate power calculations when planning the study. The outcomes studied in Paper II-III were both analyses of secondary end points in previously performed RCTs and the original sample sizes were calculated based on the primary outcomes of these studies. If the effect of vitamin D on the secondary end points were smaller than the assumed effect of vitamin D on the primary end points, then the studies may have been underpowered, increasing the risk of a false negative result. Likewise, it cannot be excluded that the null-finding in subgroups based on baseline and end-of-study s-25(OH)D in Paper II-III were caused by a type II error. Unless a trial has been designed to investigate a specific treatment effect or to distinguish a treatment effects across specific subgroups the risk of performing a type II error increases when such analyses are performed (191). Thus, the results of subgroup analyses warrants cautious interpretation, and should be considered as hypothesis generating, rather than as evidence of causality – or the lack of it.

5.2 Discussion of main findings

In the following, sleep duration and daytime sleepiness will be referred to independent of the broader entity "sleep quality", which includes insomnia and other outcomes used to define sleep quality as a construct in previous literature.

5.2.1 Association between s-25(OH)D and self-reported sleep measures

In Paper I, a tenuous association was found between s-25(OH)D and self-reported sleep measures, but the results conflicted according to sex, method of analysis, and sleep measure studied.

Although the results were mixed regarding sleep duration and ISD in Paper I, a positive association with sleep duration is supported by most previous observational studies (110;112;117-119;192;193). Null findings have also been reported (113;120;121). Higher odds of poor sleep quality with higher s-25(OH)D, as found for women in Paper I, has been reported by only a few previous studies (121;128;194), including both men and women. On the contrary, most studies have reported a lower odds of poor sleep quality with higher s-25(OH)D in both sexes (108;109;113;116;192;195-197). Null findings have also been reported (111;193;198). Finally, neither EDS, nor ESS-scores were significantly associated with s-25(OH)D in Paper I, except in women with high s-25(OH)D, which had significantly lower ESS-scores compared to women with sufficient s-25(OH)D. An inverse association with daytime sleepiness has previously been reported by some researchers (108;113;199), whereas others have reported no association between s-25(OH)D and ESS-scores (114;115).

The diverging results could be related to several factors, such as the size and type of the included sample. Except one study including 657 participants (110), all the studies reporting the association between s-25(OH)D and sleep duration, included more than 1000 participants. Thus, with samples ranging from 1,045 to more than 50,000 participants in studies finding a positive association, and from 2,459 to almost 14,500 participants in studies reporting no association, the sample size seemed to be of less importance regarding this outcome. For the relationship between s-25(OH)D and sleep quality, specific trends were difficult to discern due to the wide range of populations and sample sizes included. However, large (> 1000) population-based samples (from 18 to 84 years) have been included in studies reporting no association (193;198), as well as in studies reporting a negative association (113;192;195). In studies reporting an inverse association between sleep quality and s-25(OH)D, two (128;194)

were done in postmenopausal women. In contrast, studies done in pregnant women have been less consistent; *Cheng et al.* (116) reported higher odds of poor sleep quality with plasma 25(OH)D levels < 50 nmol/L in first-trimester pregnant women, while *Gunduz et al.* (111) found no association between s-25(OH)D and poor sleep quality in last-trimester pregnant women. Although vitamin D might be of greater significance early in pregnancy than late, the null finding by *Gunduz et al.* could also have been related to the small sample size (n=92). Likewise, the study reporting no association between s-25(OH)D and daytime sleepiness (115) was also rather small (n=348), although two of the three studies reporting an inverse association for this relationship included less than 100 participants (108;199).

As previously discussed in section 5.1.2, an increased odds of insomnia in women could represent reversed causation. Due to the considerable attention in mainstream media devoted to potential extra-skeletal health effects of vitamin D, it has become a common perception in the general public that lack of vitamin D can cause a number of unfavourable health outcomes. Thus, it was considered whether women with insomnia may have been more likely to start taking a vitamin D supplement to ameliorate their symptoms. There was some evidence in Paper I to support this hypothesis; The proportion reporting to use a vitamin D supplement was significantly higher among women with insomnia compared to women without (32.8% *v.* 26.7%, p <.001), although this was also true in men (18.2% *v.* 14.1%, p <.001). The same tendency was observed regarding other vitamin D promoting variables such as the numbers taking solarium and/or having been on a recent sunny holiday, for which the link with an increased risk of insomnia were less evident. As the aim was to estimate the association between self-reported sleep measures and s-25(OH)D from all sources, the analyses in Paper I were not adjusted for vitamin D supplement use. Thus, it cannot be excluded that insomnia may have been the cause, rather than the result of the high s-25(OH)D levels.

The inconsistent findings could have been related to differential inclusion of confounding factors. Whereas some have reported unadjusted results only (121;196), a variety of potential confounders have also been evaluated, many of which were also adjusted for in Paper I (including age, sex, BMI, physical activity, smoking status, alcohol intake, marital status, economy, shift work, and relevant comorbidities such as OSAS and depression). Depression has been positively associated with both vitamin D deficiency (200) and poor sleep quality (201;202). Only two studies reporting a positive association between s-25(OH)D and sleep quality reported to have adjusted for depression (116;195), in which statistical significance

was lost upon adjustment in one of these studies (195). However, the effect of depression on the relationship between s-25(OH)D and sleep has been questioned (203) and may vary in different populations and sleep measures. Several studies have reported heterogeneity by race for the association between s-25(OH)D and sleep (192;198;199), but findings have been contrasting; *Bertisch et al*. (198) reported a more pronounced association between s-25(OH)D and sleep duration in African Americans. *McCarty et al*. (199) found the association between s-25(OH)D and EDS to be restricted only to African Americans. In contrast, *Beydoun et al*. (192) found an association between s-25(OH)D and both sleep duration and quality among Whites only. Due to the homogenous study population of Paper I, consisting almost exclusively of Caucasians, extrapolation of these findings to other ethnic groups may not be appropriate.

The methods used to assess vitamin D status and sleep may have contributed to the observed discrepancies. Only four studies having explored the association between s-25(OH)D and sleep measures (111;112;116;198) used the gold standard LC-MS/MS method (applied in Paper I) to assess s-25(OH)D. Whereas two of these studies reported a positive association with (objective) sleep duration (112;198), two reported higher (subjective) sleep quality with higher s-25(OH)D (116;195). Objective measures of sleep duration have been positively associated with s-25(OH)D in most studies (110;112;193;198), in which s-25(OH)D was measured either using an immunoassay (110;193) or the gold standard LC-MS/MS method (112;198). The only study to not find an association (194) estimated vitamin D status based on food frequency questionnaires. When compared to objective sleep measurements, individuals with poor self-reported sleep quality have been shown to significantly underestimate total sleep time, and to overestimate SOL and the number of awakenings after sleep onset (68). In Paper I, ISD and EDS were both poorly correlated with insomnia; Among women with insomnia, only 54.1% also reported short sleep duration (<7 h) and only 12.5% also reported EDS (unpublished results). Interestingly, studies reporting both objective (i.e., PSG or actigraphy) and subjective measures of sleep duration have shown an association with s-25(OH)D with the objective measure only (193;198). The cut-offs used to differentiate between normal and non-normal sleep duration have also varied substantially between studies.

In summary, the relationship between vitamin D and self-reported sleep measures has been reported with substantial variation in findings across studies and sleep measures. The findings of Paper I support that the association is controversial. Differing study populations and

methodology complicates comparison of results across studies. In general, the reported effect estimates have been modest, with most pointing to a slight benefit of higher levels of vitamin D. The potential benefit (or harm) from vitamin D supplementation would likely be small in the general population.

5.2.2 Effect of vitamin D supplementation on self-reported sleep measures

In Paper II, we were not able to demonstrate any statistically significant effects of vitamin D supplementation on self-reported sleep measures among individuals with insufficient s-25(OH)D at baseline.

A limited number of vitamin D supplementation studies have been performed. The findings of Paper II contrasted with most previous studies, in which vitamin D has been reported to increase sleep duration (105;126) and improve sleep quality (126;127;204). Among these four, three studies applied a double-blind RCT study design (126;127;204). Neither of these studies matched the size and population included in Paper II; *Majid et al*. (126) included 89 individuals with sleep disorders aged 20-50 years old; *Ghaderi et al*. (127) included 68 individuals in maintenance methadone treatment aged 25-70 years old; *Sharifan et al*. (204) included 29 individuals with abdominal obesity aged 30-50 years old. Comparison with our findings were also complicated by the differing supplementation regimes; Whereas two of the studies gave vitamin D as an oral supplement (pearls or capsules) containing 50,000 IU once in a fourth night for 8 weeks (126) and 12 weeks (127), the latter study by *Sharifan et al*. gave low-fat milk or yoghurt fortified with 1,500 IU Nano encapsulated vitamin D3/serving. In contrast, another RCT by *Mirzaei et al*. (106), including 79 fibromyalgia patients with hypovitaminosis D (defined as s-25(OH)D < 30 ng/ml or < 75 nmol/L), found no difference in sleep quality compared to placebo using an oral supplement with vitamin D of 50,000 IU weekly for 8 weeks.

Interestingly, the largest and longest-running RCT with vitamin D (128), including 218 post-menopausal women randomized to oral vitamin D3 (2000 IU/day) for 12 months, found that repletion of s-25(OH)D ≥ 32 ng/mL (≥ 80 nmol/L) and a greater magnitude change in s-25(OH)D within the vitamin D group were associated with worse sleep quality and an increased need of sleep medications in the treatment arm of the study. However, when comparing the vitamin D group to the placebo group, there was no differences regarding these variables (128), which supported the findings of Paper II.

The null findings in Paper II could have been related to the assessment and choice of sleep variables. In particular, it was not collected objective sleep measures to complement the self-reported sleep measures in Paper I. Contrasting with all but one (204) of the previous intervention studies, the self-reported sleep measures in Paper II were not collected using the Pittsburgh Sleep Quality Index (PSQI) (51). Although the PSQI questionnaire does contain similar items (as shown in Appendix 3), this complicates comparison of Paper II with previous studies. It cannot be excluded that additional information obtained using the PSQI (e.g., afflictions of night sweat) could have changed the results of the present study. Nevertheless, based on the findings of the observation study in Paper I, this was considered unlikely.

As previously discussed, an evident shortcoming of Paper II was that the RCT was not originally designed to evaluate the effect of vitamin D supplementation on self-reported sleep measures. The major implication of this shortcoming was that the sleep questionnaire was implemented half-way into the recruitment period, which compromised the sample size. The number of participants reporting a change of sleep status (i.e., developed or recovered from ISD, insomnia and EDS) were small and likely to have been underpowered. When defined according to changes in the continuous sleep variables (i.e., sleep duration and ESS-score), the numbers were slightly larger. Using these data in an exploratory analysis showed that there were significantly more participants in the vitamin D group who reported to sleep longer, compared to the placebo group (n=47 vs. n=35, p=.018) (unpublished results). This difference did not reach statistical significance in sex-stratified analyses.

An important strength of Paper II was that the population was selected based on low s-25(OH)D at baseline (173). Three of the previous RCTs reporting a positive effect of vitamin D on self-reported sleep measures also reported baseline s-25(OH)D values below 50 nmol/L (106;127;204). However, these were all small-scale trials including less than 100 participants each. The only previous RCT of a comparable size (128) reported a baseline s-25(OH)D level of 21.4 ± 5.1 ng/mL (or 53.4 nmol/L) and found no differences between the vitamin D and the placebo group after 12 months intervention.

In summary, there were no statistically significant effects of vitamin D supplementation on self-reported sleep measures in Paper II. The evidence of a causal relationship between s-25(OH)D and self-reported sleep measures remains controversial.

5.2.3 Effect of vitamin D supplementation on BMD

In Paper III, vitamin D supplementation compared to placebo had a small positive effect on femoral neck BMD in men. A positive effect was also seen on the total hip measurement site but did not reach statistical significance compared to the placebo group. In women, there were no statistically significant differences in the effect on BMD between the vitamin D and placebo groups.

The relationship between vitamin D and BMD has been thoroughly investigated over the past decades and a vast number of RCTs have been performed. Overall, the majority of RCTs have failed to demonstrate a significant benefit of vitamin D supplementation without additional calcium in unselected populations. In the most recent meta-analysis, *Bolland et al.* (156) concluded that vitamin D supplementation alone did not "have meaningful effects on BMD", despite finding that BMD increased in three of the five reported measurement sites. The conclusion by *Bolland et al*. (156) was based on a prespecified futility boundary, which has later been criticized for being "subjectively defined" (205). To require a minimum increase in BMD for vitamin D to be considered efficient may seem unreasonable, as attenuated bone loss would be a beneficial outcome over time. Nevertheless, as pointed out by *Bolland et al.* (206), a difference compared to placebo that does not significantly counteract the average loss of BMD in post-menopausal women (from 0.5 to 1.0% a year) would be of little clinical relevance. Interestingly, the femoral neck measurement site was not among the sites to reach statistical significance (contrasting with the findings of Paper III).

Since the meta-analysis by *Bolland et al*. (156) was performed, the results of two large-scale RCT have been published. In a double-blind RCT including 399 men and women (from 40 to 80 years of age) with low baseline s-25(OH)D levels (13.6 ng/ml or 34 nmol/L), *Jorde et al*. (207) reported no effects on BMD from vitamin D 20,000 IU/week vs. placebo. Although the 4-month intervention period may not have been a sufficient to detect statistically significant effects on BMD, the study by *Jorde et al*. (207) was supported by another well-designed, large-scale double-blind RCT, namely the VITamin D and OmegA-3 TriaL (VITAL): Effects on Bone Structure and Architecture (208). The VITAL study was an ancillary study that included 771 men and women (aged ≥50 and ≥55 years respectively) from a generally healthy U.S. population. After 2 years, supplementation with vitamin D3 (2000 IU/day) showed no statistically significant effect compared to placebo in neither spine, femoral neck, total hip, nor whole body BMD.

Contrasting with the study by *Jorde et al*. (207), the participants in the VITAL study were not selected for vitamin D insufficiency. At baseline, mean total s-25(OH)D (measured using LC-MS/MS) in the VITAL study was similar to that of Paper III (levels being 69.1 nmol/L and 59.7 nmol/L, respectively). As beneficial effects of vitamin D might be restricted to, or more pronounced in, individuals with low s-25(OH)D levels, the inclusion of vitamin D sufficient individuals may have diluted the effect of vitamin D supplementation in these studies. In addition, 42.3% of the VITAL participants reported to use a vitamin D supplement up to 800 IU/day at baseline, which may have contributed to dilute a potential effect of vitamin D supplementation. In the meta-analysis by *Bolland et al*. (156), individuals with s-25(OH)D levels < 10 ng/ml (or < 25 nmol/L) achieved a statistically significant increase in lumbar BMD with daily doses of 400 IU and 1000 IU, and in hip BMD with a daily dose of 1000 IU. Subgroup analyses according to baseline s-25(OH)D showed no difference in BMD between the vitamin D and the placebo group, neither in the VITAL study (< 75, < 50, < 37 or < 30 nmol/L), nor in Paper III (< 50 nmol/L). However, neither of these analyses had been pre-specified and the number of participants with baseline mean s-25(OH)D < 50 nmol/L were limited (18% and 31.6% of the participants in the VITAL study and in Paper III, respectively). Interestingly, the VITAL study found that baseline free s-25(OH)D levels was better at predicting improvement in BMD at the spine and total hip compared to total s-25(OH)D. This finding corroborates with the free hormone hypothesis (25) but must be interpreted with caution given the number of performed comparisons in the VITAL study.

In Paper III, a positive effect of vitamin D supplementation was statistically significant only at the femoral neck measurement site. The femoral neck contains more cortical bone than what is included in the total hip measurement. Given its higher metabolic activity and larger surface area per unit volume, trabecular bone is likely more responsive to treatment than cortical bone. This has also been supported by previous studies (209). However, in the case of vitamin D deficiency, the secondary hyperparathyroidism causes bone loss mainly at cortical sites (210). Suppression of PTH (as was seen in the vitamin D group in Paper III) may thus have contributed to the attenuated loss in BMD at the femoral neck measurement site.

The effect of vitamin D supplementation on femoral neck BMD in Paper III was not observed in both sexes. Comparison of this finding with previous studies is compromised by the limited number of comparable RCTs that also included men. In general, sex differences in skeletal physiology are well-documented and supposedly regulated by androgens and estrogen (211). Given the fact that men have greater trabecular bone volume than women (211), this may

have contributed to the finding of an effect in men only in Paper III. Yet, the VITAL study found no differences in BMD according to sex in stratified analyses, neither in qualitative bone changes as measured using peripheral quantitative computed tomography (pQCT), such as change in bone structure (total, cortical, and trabecular volumetric BMD, cortical thickness) or strength (polar stress strength index, bone strength index).

As discussed regarding Paper II, the RCT constituting the fundament of Paper III were not originally designed to evaluate the effect of vitamin D alone on BMD. The study included men and women between the age of 38-80 years diagnosed with prediabetes (as described in the methods section). Thus, the inclusion criteria favoured individuals with high BMI, which would also be expected to have higher BMD due to mechanical loading. This was reflected in the small number of subjects presenting with osteopenic T-values in Paper III, in which a more prominent effect of supplementation would be expected. As presented in the original paper, a multiple logistic regression analysis (adjusted for age and BMI) was performed to evaluate the differences between the vitamin D and the placebo group regarding the incidence of fractures. This analysis ought to be interpreted with the outmost caution due to the population sample, limited power and uncertainty associated with registration of fractures.

In summary, the findings of Paper III indicated a small positive effect of vitamin D without additional calcium supplementation on femoral neck BMD in men. However, recent large-scale RCTs have not been able to confirm these results. Given that the effect estimates in Paper III were small, the clinical implications of these findings may be of modest importance.

6 Conclusions and implications for future studies

- Considering the large sample size, moderate ORs and substantial adjustment for confounding factors performed in Paper I, an association between s-25(OH)D and self-reported sleep measures (if any) is at best small in the general population. The findings of previous large-scale observational studies in unselected populations support that the association is controversial.
- The findings of Paper II do not support an effect of vitamin D supplementation on self-reported sleep measures. However, the RCT was likely underpowered to detect changes in the assessed sleep measures, as these were all secondary outcomes in the original RCT used in Paper II. Larger RCTs, specifically designed to investigate the effect of vitamin D on sleep, would be required to firmly establish the presence and size of such an effect, if one exists.
- Considering the substantial amount of RCTs and meta-analyses that have been performed, it is reasonable to state that there is enough data to conclude that an effect of vitamin D on femoral neck BMD is at best small. As suggested from the results in Paper III, it is possible that the effect is more pronounced in men compared to women, but this finding remains controversial.

References

1. Deluca HF. *History of the discovery of vitamin D and its active metabolites.* Bonekey Rep 2014. **3**:479.
2. Rejnmark L, Bislev LS, Cashman KD, Eiriksdottir G, Gaksch M, Grubler M, et al. *Non-skeletal health effects of vitamin D supplementation: A systematic review on findings from meta-analyses summarizing trial data.* PloS One 2017. **12**(7):e0180512.
3. Bouillon R, Marcocci C, Carmeliet G, Bikle D, White JH, Dawson-Hughes B, et al. *Skeletal and Extraskeletal Actions of Vitamin D: Current Evidence and Outstanding Questions.* Endocr Rev 2019. **40**(4):1109-51.
4. Bikle DD. *Extraskeletal actions of vitamin D.* Ann N Y Acad Sci 2016. **1376**(1): p. 29-52.
5. Gominak SC, Stumpf WE. *The world epidemic of sleep disorders is linked to vitamin D deficiency.* Medi Hypotheses 2012. **79**(2):132-5.
6. McCarty DE, Chesson AL, Jr., Jain SK, Marino AA. *The link between vitamin D metabolism and sleep medicine.* Sleep Med Rev 2014. **18**(4):311-9.
7. Pardridge WM, Sakiyama R, Coty WA. *Restricted transport of vitamin D and A derivatives through the rat blood-brain barrier.* J Neurochem 1985. **44**(4):1138-41.
8. Saper CB, Scammell TE, Lu J. *Hypothalamic regulation of sleep and circadian rhythms.* Nature 2005. **437**(7063):1257-63.
9. Eyles DW, Smith S, Kinobe R, Hewison M, McGrath JJ. *Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain.* J Chem Neuroanat 2005. **29**(1):21-30.
10. El-Atifi M, Dreyfus M, Berger F, Wion D. *Expression of CYP2R1 and VDR in human brain pericytes: the neurovascular vitamin D autocrine/paracrine model.* Neuroreport 2015. **26**(5):245-8.
11. Landel V, Stephan D, Cui X, Eyles D, Feron F. *Differential expression of vitamin D-associated enzymes and receptors in brain cell subtypes.* J Steroid Biochem Mol Biol 2018. **177**:129-34.
12. Holick MF. *Resurrection of vitamin D deficiency and rickets.* J Clin Invest 2006. **116**(8):2062-72.
13. Holick MF, MacLaughlin JA, Clark MB, Holick SA, Potts JT, Jr., Anderson RR, et al. *Photosynthesis of previtamin D3 in human skin and the physiologic consequences.* Science 1980. **210**(4466):203-5.
14. O'Neill CM, Kazantzidis A, Ryan MJ, Barber N, Sempos CT, Durazo-Arvizu RA, et al. *Seasonal Changes in Vitamin D-Effective UVB Availability in Europe and Associations with Population Serum 25-Hydroxyvitamin D.* Nutrients 2016. **8**(9):533.
15. Engelsen O, Brustad M, Aksnes L, Lund E. *Daily Duration of Vitamin D Synthesis in Human Skin with Relation to Latitude, Total Ozone, Altitude, Ground Cover, Aerosols and Cloud Thickness.* Photochem Photobiol 2005. **81**(6):1287-90.
16. Webb AR. *Who, what, where and when-influences on cutaneous vitamin D synthesis.* Prog Biophys Mol Biol 2006. **92**(1):17-25.
17. Gallagher JC. *Vitamin D and aging.* Endocrinol Metab Clin North Am 2013. **42**(2):319-32.
18. Sollid ST, Hutchinson MY, Fuskevåg OM, Joakimsen RM, Jorde R. *Large Individual Differences in Serum 25-Hydroxyvitamin D Response to Vitamin D Supplementation: Effects of Genetic Factors, Body Mass Index, and Baseline Concentration. Results from a Randomized Controlled Trial.* Horm Metab Res 2016. **48**(1):27-34.

19. Mazahery H, von Hurst PR. *Factors Affecting 25-Hydroxyvitamin D Concentration in Response to Vitamin D Supplementation.* Nutrients 2015. **7**(7):5111-42.
20. Sempos CT, Heijboer AC, Bikle DD, Bollerslev J, Bouillon R, Brannon PM, et al. *Vitamin D assays and the definition of hypovitaminosis D: results from the First International Conference on Controversies in Vitamin D.* Br J Clin Pharmacol 2018. **84**(10):2194-207.
21. Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. *Vitamin D: Metabolism, Molecular Mechanism of Action, and Pleiotropic Effects.* Physiol Rev 2016. **96**(1):365-408.
22. Bikle DD, Malmstroem S, Schwartz J. *Current Controversies: Are Free Vitamin Metabolite Levels a More Accurate Assessment of Vitamin D Status than Total Levels?* Endocrinol Metab Clin North Am 2017. **46**(4):901-18.
23. Nykjaer A, Dragun D, Walther D, Vorum H, Jacobsen C, Herz J, et al. *An endocytic pathway essential for renal uptake and activation of the steroid 25-(OH) vitamin D3.* Cell 1999. **96**(4):507-15.
24. Nielsen R, Christensen EI, Birn H. *Megalin and cubilin in proximal tubule protein reabsorption: from experimental models to human disease.* Kidney Int 2016. **89**(1): p. 58-67.
25. Jorde R, Grimnes G. *Serum cholecalciferol may be a better marker of vitamin D status than 25-hydroxyvitamin D.* Med Hypotheses 2018. **111**:61-5.
26. Sempos CT, Vesper HW, Phinney KW, Thienpont LM, Coates PM. *Vitamin D status as an international issue: national surveys and the problem of standardization.* Scand J Clin Lab Invest Suppl 2012. **243**:32-40.
27. Carter GD, Berry J, Durazo-Arvizu R, Gunter E, Jones G, Jones J, et al. *Hydroxyvitamin D assays: An historical perspective from DEQAS.* J Steroid Biochem Mol Biol 2018. **177**:30-5.
28. Amrein K, Scherkl M, Hoffmann M, Neuwersch-Sommeregger S, Köstenberger M, Tmava Berisha A, et al. *Vitamin D deficiency 2.0: an update on the current status worldwide.* Eur J Clin Nutr 2020. **74**(11):1498-513.
29. Cashman KD, Dowling KG, Skrabakova Z, Gonzalez-Gross M, Valtuena J, De Henauw S, et al. *Vitamin D deficiency in Europe: pandemic?* Am J Clin Nutr 2016. **103**(4):1033-44.
30. Lips P, Cashman KD, Lamberg-Allardt C, Bischoff-Ferrari HA, Obermayer-Pietsch B, Bianchi ML, et al. *Current vitamin D status in European and Middle East countries and strategies to prevent vitamin D deficiency: a position statement of the European Calcified Tissue Society.* Eur J Endocrinol 2019. **180**(4):23-54.
31. Pilz S, Zittermann A, Trummer C, Theiler-Schwetz V, Lerchbaum E, Keppel MH, et al. *Vitamin D testing and treatment: a narrative review of current evidence.* Endocr Connect 2019. **8**(2):R27-R43.
32. Holvik K, Meyer HE, Haug E, Brunvand L. *Prevalence and predictors of vitamin D deficiency in five immigrant groups living in Oslo, Norway: the Oslo Immigrant Health Study.* Eur J Clin Nutr 2005. **59**(1):57-63.
33. Holten-Andersen MN, Haugen J, Oma I, Strand TA. *Vitamin D Status and Its Determinants in A Paediatric Population in Norway.* Nutrients 2020. **12**(5):1385.
34. Oberg J, Jorde R, Almås B, Emaus N, Grimnes G. *Vitamin D deficiency and lifestyle risk factors in a Norwegian adolescent population.* Scand J Public Health 2014. **42**(7):593-602.
35. Holvik K, Brunvand L, Brustad M, Meyer HE. *Vitamin D status in the Norwegian Population.* In: Bjertness E, ed. Solar Radiat Human Health. Oslo: The Norwegian Academy of Science and Letters 2008. 216-28.

36. Giustina A, Bouillon R, Binkley N, Sempos C, Adler RA, Bollerslev J, et al. *Controversies in Vitamin D: A Statement From the Third International Conference.* JBMR Plus 2020. **4**(12):e10417.
37. Nordic Council of Ministers. *Nordic Nutrition Recommendations 2012: integrating nutrition and physical activity.* 5th ed. Copenhagen: Nordic Council of Ministers 2014.
38. Sanders KM, Stuart AL, Williamson EJ, Simpson JA, Kotowicz MA, Young D, et al. *Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial.* JAMA 2010. **303**(18):1815-22.
39. Amrein K, Papinutti A, Mathew E, Vila G, Parekh D. *Vitamin D and critical illness: what endocrinology can learn from intensive care and vice versa.* Endocr Connect 2018. **7**(12):R304-R15.
40. Zittermann A, Prokop S, Gummert JF, Börgermann J. *Safety issues of vitamin D supplementation.* Anticancer Agents Med Chem 2013. **13**(1):4-10.
41. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. *Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline.* J Clin Endocrinol Metab 2011. **96**(7):1911-30.
42. Melamed ML, Michos ED, Post W, Astor B. *25-hydroxyvitamin D levels and the risk of mortality in the general population.* Arch Intern Med 2008. **168**(15):1629-37.
43. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. *The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know.* J Clin Endocrinol Metab 2011. **96**(1):53-8.
44. EFSA Panel on Dietetic Products N, Allergies. *Scientific Opinion on the Tolerable Upper Intake Level of vitamin D.* EFSA Journal 2012. **10**(7):2813. Available from: https://www.efsa.europa.eu/en/efsajournal/pub/2813.
45. World Health Organization Regional Office for Europe and the European Centre for Environment and Health Bonn Office. *Report of the WHO technical meeting on sleep and health* [Internet]. Bonn, Germany: 2004 [cited 2021 Sept 17]. Avaliable from: https://www.ilo.org/safework/areasofwork/WCMS_118388/lang--en/index.htm.
46. Hayley AC, Sivertsen B, Hysing M, Vedaa Ø, Øverland S. *Sleep difficulties and academic performance in Norwegian higher education students.* Br J Educ Psychol 2017. **87**(4):722-37.
47. Baglioni C, Nanovska S, Regen W, Spiegelhalder K, Feige B, Nissen C, et al. *Sleep and mental disorders: A meta-analysis of polysomnographic research.* Psychol Bull 2016. **142**(9):969-90.
48. Walker WH, 2nd, Walton JC, DeVries AC, Nelson RJ. *Circadian rhythm disruption and mental health.* Transl Psychiatry 2020. **10**(1):28.
49. Sivertsen B, Lallukka T, Salo P, Pallesen S, Hysing M, Krokstad S, et al. *Insomnia as a risk factor for ill health: results from the large population-based prospective HUNT Study in Norway.* J Sleep Res 2014. **23**(2):124-32.
50. Hirshkowitz M, Whiton K, Albert SM, Alessi C, Bruni O, DonCarlos L, et al. *National Sleep Foundation's updated sleep duration recommendations: final report.* Sleep Health 2015. **1**(4):233-43.
51. Watson NF, Badr MS, Belenky G, Bliwise DL, Buxton OM, Buysse D, et al. *Recommended Amount of Sleep for a Healthy Adult: A Joint Consensus Statement of the American Academy of Sleep Medicine and Sleep Research Society.* Sleep 2015. **38**(6):843-4.
52. Barnes CM, Drake CL. *Prioritizing Sleep Health: Public Health Policy Recommendations.* Perspect Psychol Sci 2015. **10**(6):733-7.

53. Stranges S, Tigbe W, Gómez-Olivé FX, Thorogood M, Kandala NB. *Sleep problems: an emerging global epidemic? Findings from the INDEPTH WHO-SAGE study among more than 40,000 older adults from 8 countries across Africa and Asia.* Sleep 2012. **35**(8):1173-81.
54. Hillman D, Mitchell S, Streatfeild J, Burns C, Bruck D, Pezzullo L. *The economic cost of inadequate sleep.* Sleep 2018. **41**(8):1-13.
55. Hafner M, Stepanek M, Taylor J, Troxel WM, van Stolk C. *Why Sleep Matters-The Economic Costs of Insufficient Sleep: A Cross-Country Comparative Analysis.* Rand Health Q 2017. **6**(4):11.
56. Léger D, Bayon V. *Societal costs of insomnia.* Sleep Med Rev 2010. **14**(6):379-89.
57. Kessler RC, Berglund PA, Coulouvrat C, Hajak G, Roth T, Shahly V, et al. *Insomnia and the performance of US workers: results from the America insomnia survey.* Sleep 2011. **34**(9):1161-71.
58. Hysing M, Harvey AG, Linton SJ, Askeland KG, Sivertsen B. *Sleep and academic performance in later adolescence: results from a large population-based study.* J Sleep Res 2016. **25**(3):318-24.
59. Hysing M, Haugland S, Stormark KM, Bøe T, Sivertsen B. *Sleep and school attendance in adolescence: results from a large population-based study.* Scand J Public Health 2015. **43**(1):2-9.
60. Sivertsen B, Overland S, Bjorvatn B, Maeland JG, Mykletun A. *Does insomnia predict sick leave? The Hordaland Health Study.* J Psychosom Res 2009. **66**(1):67-74.
61. Sivertsen B, Overland S, Neckelmann D, Glozier N, Krokstad S, Pallesen S, et al. *The long-term effect of insomnia on work disability: the HUNT-2 historical cohort study.* Am J Epidemiol 2006. **163**(11):1018-24.
62. Pallesen S, Sivertsen B, Nordhus IH, Bjorvatn B. *A 10-year trend of insomnia prevalence in the adult Norwegian population.* Sleep Med 2014. **15**(2):173-9.
63. Sivertsen B, Pallesen S, Friborg O, Nilsen KB, Bakke Ø K, Goll JB, et al. *Sleep patterns and insomnia in a large population-based study of middle-aged and older adults: The Tromsø study 2015-2016.* J Sleep Res 2021. **30**(1):e13095.
64. Neikrug AB, Ancoli-Israel S. *Sleep disorders in the older adult - a mini-review.* Gerontology 2010. **56**(2):181-9.
65. Crowley K. *Sleep and sleep disorders in older adults.* Neuropsychol Rev 2011. **21**(1):41-53.
66. Li J, Vitiello MV, Gooneratne NS. *Sleep in Normal Aging.* Sleep Med Clin 2018. **13**(1):1-11.
67. Mong JA, Cusmano DM. *Sex differences in sleep: impact of biological sex and sex steroids.* Philos Trans R Soc Lond B Biol Sci 2016. **371**(1688):20150110.
68. Trimmel K, Eder HG, Böck M, Stefanic-Kejik A, Klösch G, Seidel S. *The (mis)perception of sleep: factors influencing the discrepancy between self-reported and objective sleep parameters.* J Clin Sleep Med 2021. **17**(5):917-24.
69. Brown JP, Martin D, Nagaria Z, Verceles AC, Jobe SL, Wickwire EM. *Mental Health Consequences of Shift Work: An Updated Review.* Curr Psychiatry Rep 2020. **22**(2):7.
70. Rognmo K, Bergvik S, Rosenvinge JH, Bratlid KL, Friborg O. *Gender differences in the bidirectional relationship between alcohol consumption and sleeplessness: the Tromsø study.* BMC Public Health 2019. **19**(1):444.
71. American Academy of Sleep Medicine. *International Classification of Sleep Disorders: ICSD-3.* 3rd ed. Darien, IL: American Academy of Sleep Medicine 2014.
72. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders: DSM-5.* 5th ed. Washington, D.C: American Psychiatric Association 2013.

73. Thorpy M. International Classification of Sleep Disorders. In: Sleep Disorders Medicine: New York, NY: Springer New York 2017. 475-84.
74. Ohayon M, Wickwire EM, Hirshkowitz M, Albert SM, Avidan A, Daly FJ, et al. *National Sleep Foundation's sleep quality recommendations: first report.* Sleep Health 2017. **3**(1):6-19.
75. Ramlee F, Afolalu EF, Tang NKY. *Do People With Chronic Pain Judge Their Sleep Differently? A Qualitative Study.* Behav Sleep Med 2018. **16**(3):259-71.
76. Markun LC, Sampat A. Clinician-Focused Overview and Developments in Polysomnography. Curr Sleep Med Rep 2020:1-13.
77. Edinger JD, Fins AI, Sullivan RJ, Jr., Marsh GR, Dailey DS, Hope TV, et al. *Sleep in the laboratory and sleep at home: comparisons of older insomniacs and normal sleepers.* Sleep 1997. **20**(12):1119-26.
78. Henriksen A, Haugen Mikalsen M, Woldaregay AZ, Muzny M, Hartvigsen G, Hopstock LA, et al. *Using Fitness Trackers and Smartwatches to Measure Physical Activity in Research: Analysis of Consumer Wrist-Worn Wearables.* J Med Internet Res 2018. **20**(3):e110.
79. Berger AM, Wielgus KK, Young-McCaughan S, Fischer P, Farr L, Lee KA. *Methodological challenges when using actigraphy in research.* J Pain Symptom Manage 2008. **36**(2):191-9.
80. Krystal AD, Edinger JD. *Measuring sleep quality.* Sleep Med 2008. **9**:S10-S7.
81. Akerstedt T, Hume K, Minors D, Waterhouse J. *Good sleep--its timing and physiological sleep characteristics.* J Sleep Res 1997. **6**(4):221-9.
82. Rezaie L, Fobian AD, McCall WV, Khazaie H. *Paradoxical insomnia and subjective-objective sleep discrepancy: A review.* Sleep medicine reviews 2018. **40**:196-202.
83. Matthews KA, Patel SR, Pantesco EJ, Buysse DJ, Kamarck TW, Lee L, et al. *Similarities and differences in estimates of sleep duration by polysomnography, actigraphy, diary, and self-reported habitual sleep in a community sample.* Sleep Health 2018. **4**(1):96-103.
84. Lauderdale DS, Knutson KL, Yan LL, Liu K, Rathouz PJ. *Self-reported and measured sleep duration: how similar are they?* Epidemiology 2008. **19**(6):838-45.
85. Cespedes EM, Hu FB, Redline S, Rosner B, Alcantara C, Cai J, et al. *Comparison of Self-Reported Sleep Duration With Actigraphy: Results From the Hispanic Community Health Study/Study of Latinos Sueño Ancillary Study.* Am J Epidemiol 2016. **183**(6):561-73.
86. Holmøy T, Moen SM. *Assessing vitamin D in the central nervous system.* Acta Neurol Scand 2010. **122**(Suppl. 190):88-92.
87. Fu X, Dolnikowski GG, Patterson WB, Dawson-Hughes B, Zheng T, Morris MC, et al. *Determination of Vitamin D and Its Metabolites in Human Brain Using an Ultra-Pressure LC-Tandem Mass Spectra Method.* Curr Dev Nutr 2019. **3**(7):nzz074.
88. Stumpf WE, O'Brien LP. *1,25 (OH)2 vitamin D3 sites of action in the brain. An autoradiographic study.* Histochemistry 1987. **87**(5):393-406.
89. Stumpf WE, Bidmon HJ, Li L, Pilgrim C, Bartke A, Mayerhofer A, et al. *Nuclear receptor sites for vitamin D-soltriol in midbrain and hindbrain of Siberian hamster (Phodopus sungorus) assessed by autoradiography.* Histochemistry 1992. **98**(3):155-64.
90. Musiol IM, Stumpf WE, Bidmon HJ, Heiss C, Mayerhofer A, Bartke A. *Vitamin D nuclear binding to neurons of the septal, substriatal and amygdaloid area in the Siberian hamster (Phodopus sungorus) brain.* Neuroscience 1992. **48**(4):841-8.

91. Prüfer K, Veenstra TD, Jirikowski GF, Kumar R. *Distribution of 1,25-dihydroxyvitamin D3 receptor immunoreactivity in the rat brain and spinal cord.* J Chem Neuroanat 1999. **16**(2):135-45.
92. Cui X, Pelekanos M, Liu PY, Burne TH, McGrath JJ, Eyles DW. *The vitamin D receptor in dopamine neurons; its presence in human substantia nigra and its ontogenesis in rat midbrain.* Neuroscience 2013. **236**:77-87.
93. Romano F, Muscogiuri G, Di Benedetto E, Zhukouskaya VV, Barrea L, Savastano S, et al. *Vitamin D and Sleep Regulation: Is there a Role for Vitamin D?* Curr Pharm Des 2020. **26**(21):2492-6.
94. Muscogiuri G, Barrea L, Scannapieco M, Di Somma C, Scacchi M, Aimaretti G, et al. *The lullaby of the sun: the role of vitamin D in sleep disturbance.* Sleep Med 2019. **54**:262-5.
95. Gutierrez-Monreal MA, Cuevas-Diaz Duran R, Moreno-Cuevas JE, Scott SP. *A role for 1α,25-dihydroxyvitamin d3 in the expression of circadian genes.* J Biol Rhythms 2014. **29**(5):384-8.
96. Ghareghani M, Reiter RJ, Zibara K, Farhadi N. *Latitude, Vitamin D, Melatonin, and Gut Microbiota Act in Concert to Initiate Multiple Sclerosis: A New Mechanistic Pathway.* Front Immunol 2018. **9**:2484.
97. Annweiler C, Beauchet O, Bartha R, Graffe A, Milea D, Montero-Odasso M. *Association Between Serum 25-Hydroxyvitamin D Concentration and Optic Chiasm Volume.* J Am Geriatr Soc 2013. **61**(6):1026-8.
98. Dibner C, Schibler U, Albrecht U. *The mammalian circadian timing system: organization and coordination of central and peripheral clocks.* Annu Rev Physiol 2010. **72**:517-49.
99. Kaneko I, Sabir MS, Dussik CM, Whitfield GK, Karrys A, Hsieh JC, et al. *1,25-Dihydroxyvitamin D regulates expression of the tryptophan hydroxylase 2 and leptin genes: implication for behavioral influences of vitamin D.* FASEB J 2015. **29**(9):4023-35.
100. Bhat A, Pires AS, Tan V, Babu Chidambaram S, Guillemin GJ. *Effects of Sleep Deprivation on the Tryptophan Metabolism.* Int J Tryptophan Res 2020. **13**:1178646920970902.
101. Calton EK, Keane KN, Newsholme P, Soares MJ. *The Impact of Vitamin D Levels on Inflammatory Status: A Systematic Review of Immune Cell Studies.* PloS One 2015. **10**(11):e0141770.
102. Müller K, Diamant M, Bendtzen K. *Inhibition of production and function of interleukin-6 by 1,25-dihydroxyvitamin D3.* Immunol Lett 1991. **28**(2):115-20.
103. Zhong A, Xiong X, Shi M, Xu H. *Roles of interleukin (IL)-6 gene polymorphisms, serum IL-6 levels, and treatment in obstructive sleep apnea: a meta-analysis.* Sleep Breath 2016. **20**(2):719-31.
104. Haack M, Sanchez E, Mullington JM. *Elevated inflammatory markers in response to prolonged sleep restriction are associated with increased pain experience in healthy volunteers.* Sleep 2007. **30**(9):1145-52.
105. Huang W, Shah S, Long Q, Crankshaw AK, Tangpricha V. *Improvement of pain, sleep, and quality of life in chronic pain patients with vitamin D supplementation.* Clin J Pain 2013. **29**(4):341-7.
106. Mirzaei A, Zabihiyeganeh M, Jahed SA, Khiabani E, Nojomi M, Ghaffari S. *Effects of vitamin D optimization on quality of life of patients with fibromyalgia: A randomized controlled trial.* Med J Islam Repub Iran 2018. **32**:29.

107. Gao Q, Kou T, Zhuang B, Ren Y, Dong X, Wang Q. *The Association between Vitamin D Deficiency and Sleep Disorders: A Systematic Review and Meta-Analysis.* Nutrients 2018. **10**(10):1395.
108. Ekinci RMK, Balci S, Serbes M, Dogruel D, Altintas DU, Yilmaz M. *Decreased serum vitamin B(12) and vitamin D levels affect sleep quality in children with familial Mediterranean fever.* Rheumatol Int 2018. **38**(1):83-7.
109. Jung YS, Chae CH, Kim YO, Son JS, Kim CW, Park HO, et al. *The relationship between serum vitamin D levels and sleep quality in fixed day indoor field workers in the electronics manufacturing industry in Korea.* Ann Occup Environ Med 2017. **29**:25.
110. Piovezan RD, Hirotsu C, Feres MC, Cintra FD, Andersen ML, Tufik S, et al. *Obstructive sleep apnea and objective short sleep duration are independently associated with the risk of serum vitamin D deficiency.* PloS one 2017. **12**(7):e0180901.
111. Gunduz S, Kosger H, Aldemir S, Akcal B, Tevrizci H, Hizli D, et al. *Sleep deprivation in the last trimester of pregnancy and inadequate vitamin D: Is there a relationship?* J Chin Med Assoc 2016. **79**(1):34-8.
112. Massa J, Stone KL, Wei EK, Harrison SL, Barrett-Connor E, Lane NE, et al. *Vitamin D and actigraphic sleep outcomes in older community-dwelling men: the MrOS sleep study.* Sleep 2015. **38**(2):251-7.
113. Beydoun MA, Gamaldo AA, Canas JA, Beydoun HA, Shah MT, McNeely JM, et al. *Serum nutritional biomarkers and their associations with sleep among US adults in recent national surveys.* PloS one 2014. **9**(8):e103490.
114. Carlander B, Puech-Cathala AM, Jaussent I, Scholz S, Bayard S, Cochen V, et al. *Low vitamin D in narcolepsy with cataplexy.* PloS one 2011. **6**(5):e20433.
115. Dauvilliers Y, Evangelista E, Lopez R, Barateau L, Scholz S, Crastes de Paulet B, et al. *Vitamin D deficiency in type 1 narcolepsy: a reappraisal.* Sleep Med 2017. **29**:1-6.
116. Cheng TS, Loy SL, Cheung YB, Cai S, Colega MT, Godfrey KM, et al. *Plasma Vitamin D Deficiency Is Associated With Poor Sleep Quality and Night-Time Eating at Mid-Pregnancy in Singapore.* Nutrients 2017. **9**(4):340.
117. Kim SY, Lee MH, Lim WJ, Kim SI, Lee YJ. *Associations of 25-Hydroxyvitamin D Levels and Arthritis with Sleep Duration: The Korean National Health and Nutrition Examination Survey 2008-2014.* Nat Sci Sleep 2020. **12**:883-94.
118. Kim JH, Chang JH, Kim DY, Kang JW. *Association between self-reported sleep duration and serum vitamin D level in elderly Korean adults.* J Am Geriatr Soc 2014. **62**(12):2327-32.
119. Doo M. *The Association between Sleep Duration and 25-Hydroxyvitamin D Concentration with Obesity in an Elderly Korean Population: A Cross-Sectional Study.* Nutrients 2018. **10**(5):575.
120. Choi JH, Lee B, Lee JY, Kim CH, Park B, Kim DY, et al. *Relationship between Sleep Duration, Sun Exposure, and Serum 25-Hydroxyvitamin D Status: A Cross-sectional Study.* Sci Rep 2020. **10**(1):4168.
121. Shiue I. *Low vitamin D levels in adults with longer time to fall asleep: US NHANES, 2005-2006.* Int J Cardiol 2013. **168**(5):5074-5.
122. Yavuz D, Demirağ MD, Yavuz R, Karagöz Özen DS, Ramazanoğlu ZB. *25-Hydroxy vitamin D level is associated with sleep disturbances in patients with chronic kidney disease on hemodialysis: a cross-sectional study.* Turk J Med Sci 2020. **50**(2):298-303.
123. Han B, Zhu FX, Shi C, Wu HL, Gu XH. *Association between Serum Vitamin D Levels and Sleep Disturbance in Hemodialysis Patients.* Nutrients 2017. **9**(2):139.

124. Heybeli C, Soysal P, Oktan MA, Smith L, Çelik A, Kazancioglu R. *Associations between nutritional factors and excessive daytime sleepiness in older patients with chronic kidney disease.* Aging Clin Exp Res 2021. Aug 27 [Epub ahead of print].
125. Chan V, Lo K. *Efficacy of dietary supplements on improving sleep quality: a systematic review and meta-analysis.* Postgrad Med J 2021. Jan 13 [Epub ahead of print].
126. Majid MS, Ahmad HS, Bizhan H, Hosein HZM, Mohammad A. *The effect of vitamin D supplement on the score and quality of sleep in 20-50 year-old people with sleep disorders compared with control group.* Nutr Neurosci 2018. **21**(7):511-9.
127. Ghaderi A, Banafshe HR, Motmaen M, Rasouli-Azad M, Bahmani F, Asemi Z. *Clinical trial of the effects of vitamin D supplementation on psychological symptoms and metabolic profiles in maintenance methadone treatment patients.* Prog Neuropsychopharmacol Biol Psychiatry 2017. **79**(Pt B):84-9.
128. Mason C, de Dieu Tapsoba J, Duggan C, Wang CY, Korde L, McTiernan A. *Repletion of vitamin D associated with deterioration of sleep quality among postmenopausal women.* Prev Med 2016. **93**:166-70.
129. Clarke B. *Normal bone anatomy and physiology.* Clin J Am Soc Nephrol 2008. **3**(Suppl 3):S131-9.
130. Taichman RS. *Blood and bone: two tissues whose fates are intertwined to create the hematopoietic stem-cell niche.* Blood 2005. **105**(7):2631-9.
131. Buck DW, 2nd, Dumanian GA. *Bone biology and physiology: Part I. The fundamentals.* Plast Reconstr Surg 2012. **129**(6):1314-20.
132. Buckwalter JA, Glimcher MJ, Cooper RR, Recker R. *Bone biology. I: Structure, blood supply, cells, matrix, and mineralization.* J Bone Joint Surg Am 1995. **77**(8): 1256-75.
133. Sommerfeldt DW, Rubin CT. *Biology of bone and how it orchestrates the form and function of the skeleton.* Eur Spine J 2001. **10**(Suppl 2):S86-95.
134. Manolagas SC. *Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis.* Endoc Rev 2000. **21**(2):115-37.
135. Henriksen K, Neutzsky-Wulff AV, Bonewald LF, Karsdal MA. *Local communication on and within bone controls bone remodeling.* Bone 2009. **44**(6):1026-33.
136. Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Boyle WJ, Riggs BL. *The roles of osteoprotegerin and osteoprotegerin ligand in the paracrine regulation of bone resorption.* J Bone Miner Res 2000. **15**(1):2-12.
137. Riggs BL, Melton LJ, Robb RA, Camp JJ, Atkinson EJ, McDaniel L, et al. *A population-based assessment of rates of bone loss at multiple skeletal sites: evidence for substantial trabecular bone loss in young adult women and men.* J Bone Miner Res 2008. **23**(2):205-14.
138. Khosla S, Melton LJ, 3rd, Riggs BL. *The unitary model for estrogen deficiency and the pathogenesis of osteoporosis: is a revision needed?* J Bone Miner Res 2011. **26**(3):441-51.
139. Marie PJ, Kassem M. *Extrinsic mechanisms involved in age-related defective bone formation.* J Clin Endocrinol Metab 2011. **96**(3):600-9.
140. Almeida M, Laurent MR, Dubois V, Claessens F, O'Brien CA, Bouillon R, et al. *Estrogens and Androgens in Skeletal Physiology and Pathophysiology.* Physiol Rev 2017. **97**(1):135-87.
141. Khosla S. *Estrogen and bone: insights from estrogen-resistant, aromatase-deficient, and normal men.* Bone 2008. **43**(3):414-7.

142. Camacho PM, Petak SM, Binkley N, Diab DL, Eldeiry LS, Farooki A, et al. *AMERICAN ASSOCIATION OF CLINICAL ENDOCRINOLOGISTS/AMERICAN COLLEGE OF ENDOCRINOLOGY CLINICAL PRACTICE GUIDELINES FOR THE DIAGNOSIS AND TREATMENT OF POSTMENOPAUSAL OSTEOPOROSIS- 2020 UPDATE EXECUTIVE SUMMARY.* Endocr Pract 2020. **26**(5):564-70.
143. Cosman F, de Beur SJ, LeBoff MS, Lewiecki EM, Tanner B, Randall S, et al. *Clinician's Guide to Prevention and Treatment of Osteoporosis.* Osteoporos Int 2014. **25**(10):2359-81.
144. Fukumoto S. *Phosphate metabolism and vitamin D.* Bonekey Rep 2014. **3**:497.
145. Aspray TJ, Bowring C, Fraser W, Gittoes N, Javaid MK, Macdonald H, et al. *National Osteoporosis Society Vitamin D Guideline Summary.* Age Ageing 2014. **43**(5):592-5.
146. van de Peppel J, van Leeuwen JP. *Vitamin D and gene networks in human osteoblasts.* Front Physiol 2014. **5**:137.
147. Anderson PH. Vitamin D Activity and Metabolism in Bone. Curr Osteoporos Rep 2017. **15**(5):443-9.
148. Lieben L, Masuyama R, Torrekens S, Van Looveren R, Schrooten J, Baatsen P, et al. *Normocalcemia is maintained in mice under conditions of calcium malabsorption by vitamin D-induced inhibition of bone mineralization.* J Clin Invest 2012. **122**(5):1803-15.
149. Ebeling PR. *Vitamin D and bone health: Epidemiologic studies.* Bonekey reports 2014. **3**:511.
150. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. *Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes.* Am J Clin Nutr 2006. **84**(1):18-28.
151. Reid IR, Bolland MJ, Grey A. *Effects of vitamin D supplements on bone mineral density: a systematic review and meta-analysis.* Lancet 2014. **383**(9912):146-55.
152. Bolland MJ, Grey A, Gamble GD, Reid IR. *The effect of vitamin D supplementation on skeletal, vascular, or cancer outcomes: a trial sequential meta-analysis.* Lancet Diabetes Endocrinol 2014. **2**(4):307-20.
153. Theodoratou E, Tzoulaki I, Zgaga L, Ioannidis JP. *Vitamin D and multiple health outcomes: umbrella review of systematic reviews and meta-analyses of observational studies and randomised trials.* BMJ 2014. **348**:g2035.
154. Avenell A, Mak JC, O'Connell D. *Vitamin D and vitamin D analogues for preventing fractures in post-menopausal women and older men.* Cochrane Database Syst Rev 2014. **4**:CD000227.
155. Zhao JG, Zeng XT, Wang J, Liu L. *Association Between Calcium or Vitamin D Supplementation and Fracture Incidence in Community-Dwelling Older Adults: A Systematic Review and Meta-analysis.* JAMA 2017. **318**(24):2466-82.
156. Bolland MJ, Grey A, Avenell A. *Effects of vitamin D supplementation on musculoskeletal health: a systematic review, meta-analysis, and trial sequential analysis.* Lancet Diabetes Endocrinol 2018. **6**(11):847-58.
157. Cameron ID, Gillespie LD, Robertson MC, Murray GR, Hill KD, Cumming RG, et al. *Interventions for preventing falls in older people in care facilities and hospitals.* Cochrane Database Sys Rev 2012. **12**:CD005465.
158. Gillespie LD, Robertson MC, Gillespie WJ, Sherrington C, Gates S, Clemson LM, et al. *Interventions for preventing falls in older people living in the community.* Cochrane Database Sys Rev 2012. **9**:CD007146.
159. Jacobsen BK, Eggen AE, Mathiesen EB, Wilsgaard T, Njolstad I. *Cohort profile: the Tromso Study.* Int J Epidemiol 2012. **41**(4):961-7.

160. Kubiak J, Thorsby PM, Kamycheva E, Jorde R. *Vitamin D supplementation does not improve CVD risk factors in vitamin D-insufficient subjects.* Endocr Connect 2018. **7**(6):840-9.
161. Sollid ST, Hutchinson MY, Fuskevag OM, Figenschau Y, Joakimsen RM, Schirmer H, et al. *No effect of high-dose vitamin D supplementation on glycemic status or cardiovascular risk factors in subjects with prediabetes.* Diabetes Care 2014. **37**(8):2123-31.
162. Jorde R, Sollid ST, Svartberg J, Schirmer H, Joakimsen RM, Njolstad I, et al. *Vitamin D 20,000 IU per Week for Five Years Does Not Prevent Progression From Prediabetes to Diabetes.* J Clin Endocrinol Metab 2016. **101**(4):1647-55.
163. Eggen AE, Mathiesen EB, Wilsgaard T, Jacobsen BK, Njølstad I. *The sixth survey of the Tromso Study (Tromso 6) in 2007-08: collaborative research in the interface between clinical medicine and epidemiology: study objectives, design, data collection procedures, and attendance in a multipurpose population-based health survey.* Scand J Public Health 2013. **41**(1):65-80.
164. Melsom T, Mathisen UD, Eilertsen BA, Ingebretsen OC, Jenssen T, Njolstad I, et al. *Physical exercise, fasting glucose, and renal hyperfiltration in the general population: the Renal Iohexol Clearance Survey in Tromso 6 (RENIS-T6).* Clin J Am Soc Nephrol 2012. **7**(11):1801-10.
165. Sneve M, Figenschau Y, Jorde R. *Supplementation with cholecalciferol does not result in weight reduction in overweight and obese subjects.* Eur J Endocrinol 2008. **159**(6):675-84.
166. Pallesen S, Bjorvatn B, Nordhus IH, Sivertsen B, Hjørnevik M, Morin CM. *A new scale for measuring insomnia: the Bergen Insomnia Scale.* Percept Mot Skills 2008. **107**(3):691-706.
167. Johns MW. *A new method for measuring daytime sleepiness: the Epworth sleepiness scale.* Sleep 1991. **14**(6):540-5.
168. Beiske KK, Kjelsberg FN, Ruud EA, Stavem K. *Reliability and validity of a Norwegian version of the Epworth sleepiness scale.* Sleep Breath 2009. **13**(1):65-72.
169. Lin M, Lucas HC, Shmueli G. *Research Commentary: Too Big to Fail: Large Samples and the p-Value Problem.* Inf Syst Res 2013. **24**(4):906-17.
170. Hill AB. *THE ENVIRONMENT AND DISEASE: ASSOCIATION OR CAUSATION?* Proc R Soc Med 1965. **58**(5):295-300.
171. Jorde R. *RCTS are the only appropriate way to demonstrate the role of vitamin D in health.* J Steroid Biochem Mol Biol 2018. **177**:10-4.
172. Scragg R. *Limitations of vitamin D supplementation trials: Why observational studies will continue to help determine the role of vitamin D in health.* J Steroid Biochem Mol Biol 2018. **177**:6-9.
173. Heaney RP. *Guidelines for optimizing design and analysis of clinical studies of nutrient effects.* Nutr Rev 2014. **72**(1):48-54.
174. Goodman SN, Berlin JA. *The use of predicted confidence intervals when planning experiments and the misuse of power when interpreting results.* Ann Inter Med 1994. **121**(3):200-6.
175. Rothman KJ, Greenland S, Lash TL. *Modern Epidemiology*. 3rd ed. Philadelphia: Wolters Kluwer 2008.
176. Elwert F, Winship C. *Endogenous Selection Bias: The Problem of Conditioning on a Collider Variable.* Annu Rev Sociol 2014. **40**:31-53.
177. Greenland S, Pearl J, Robins JM. *Causal diagrams for epidemiologic research.* Epidemiology 1999. **10**(1):37-48.

178. Janssen HC, Emmelot-Vonk MH, Verhaar HJ, van der Schouw YT. *Determinants of vitamin D status in healthy men and women aged 40-80 years.* Maturitas 2013. **74**(1):79-83.
179. Manson JE, Shufelt CL, Robins JM. *The Potential for Postrandomization Confounding in Randomized Clinical Trials.* JAMA 2016. **315**(21):2273-4.
180. Hernán MA, Hernández-Díaz S, Robins JM. *Randomized trials analyzed as observational studies.* Ann Intern Med 2013. **159**(8):560-2.
181. Rooney MR, Harnack L, Michos ED, Ogilvie RP, Sempos CT, Lutsey PL. *Trends in Use of High-Dose Vitamin D Supplements Exceeding 1000 or 4000 International Units Daily, 1999-2014.* JAMA 2017. **317**(23):2448-50.
182. Szklo M, Nieto FJ. *Epidemiology : beyond the basics.* 4th ed. Sudbury: Jones & Bartlett Learning LLC 2018.
183. Langhammer A, Krokstad S, Romundstad P, Heggland J, Holmen J. *The HUNT study: participation is associated with survival and depends on socioeconomic status, diseases and symptoms.* BMC Med Res Methodol 2012. **12**(1):143.
184. Leening MJG, Heeringa J, Deckers JW, Franco OH, Hofman A, Witteman JCM, et al. *Healthy Volunteer Effect and Cardiovascular Risk.* Epidemiology 2014. **25**(3):470-1.
185. Pallesen S, Nordhus IH, Omvik S, Sivertsen B, Tell GS, Bjorvatn B. *Prevalence and risk factors of subjective sleepiness in the general adult population.* Sleep 2007. **30**(5):619-24.
186. Lewis SC, Warlow CP. *How to spot bias and other potential problems in randomised controlled trials.* J Neurol Neurosurg Psychiatry 2004. **75**(2):181-7.
187. Connor Gorber S, Schofield-Hurwitz S, Hardt J, Levasseur G, Tremblay M. *The accuracy of self-reported smoking: a systematic review of the relationship between self-reported and cotinine-assessed smoking status.* Nicotine Tob Res 2009. **11**(1):12-24.
188. Emaus A, Degerstrøm J, Wilsgaard T, Hansen BH, Dieli-Conwright CM, Furberg AS, et al. *Does a variation in self-reported physical activity reflect variation in objectively measured physical activity, resting heart rate, and physical fitness? Results from the Tromso study.* Scand J Public Health 2010. **38**(5 Suppl):105-18.
189. Vickers A. *What is a P-value anyway? 34 stories to help you actually understand statistics.* 1st ed. Boston, MA: Addison Wesley 2010.
190. Benjamini Y, Hochberg Y. *Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing.* J R Stat Soc Series B Stat Methodol 1995. **57**(1):289-300.
191. Yusuf S, Wittes J, Probstfield J, Tyroler HA. *Analysis and interpretation of treatment effects in subgroups of patients in randomized clinical trials.* JAMA 1991. **266**(1):93-8.
192. Beydoun MA, Ng AE, Fanelli-Kuczmarski MT, Hossain S, Beydoun HA, Evans MK, et al. *Vitamin D status and its longitudinal association with changes in patterns of sleep among middle-aged urban adults.* J Affect Disord 2021. **282**:858-68.
193. Dogan-Sander E, Willenberg A, Batmaz İ, Enzenbach C, Wirkner K, Kohls E, et al. *Association of serum 25-hydroxyvitamin D concentrations with sleep phenotypes in a German community sample.* PloS One 2019. **14**(7):e0219318.
194. Grandner MA, Kripke DF, Naidoo N, Langer RD. *Relationships among dietary nutrients and subjective sleep, objective sleep, and napping in women.* Sleep Med 2010. **11**(2):180-4.
195. Nakamura K, Hui SP, Ukawa S, Okada E, Nakagawa T, Okabe H, et al. *Serum 25-hydroxyvitamin D(3) levels and poor sleep quality in a Japanese population: the DOSANCO Health Study.* Sleep Med 2019. **57**:135-40.

196. Çakır T, Doğan G, Subaşı V, Filiz MB, Ülker N, Doğan Ş K, et al. *An evaluation of sleep quality and the prevalence of restless leg syndrome in vitamin D deficiency.* Acta Neurol Belg 2015. **115**(4):623-7.
197. Song EK, Wu JR. *Associations of Vitamin D Intake and Sleep Quality With Cognitive Dysfunction in Older Adults With Heart Failure.* J Cardiovasc Nurs 2018. **33**(4):392-9.
198. Bertisch SM, Sillau S, de Boer IH, Szklo M, Redline S. *25-Hydroxyvitamin D Concentration and Sleep Duration and Continuity: Multi-Ethnic Study of Atherosclerosis.* Sleep 2015. **38**(8):1305-11.
199. McCarty DE, Reddy A, Keigley Q, Kim PY, Marino AA. *Vitamin D, race, and excessive daytime sleepiness.* J Clin Sleep Med 2012. **8**(6):693-7.
200. Kjærgaard M, Joakimsen R, Jorde R. *Low serum 25-hydroxyvitamin D levels are associated with depression in an adult Norwegian population.* Psychiatry Res 2011. **190**(2-3):221-5.
201. Fetveit A, Bjorvatn B. *[Sleep disorders in the elderly]*. Tidsskr Nor Laegeforen 2009. **129**(19):2004-6.
202. Fetveit A, Straand J, Bjorvatn B. *Sleep disturbances in an arctic population: the Tromso Study.* BMC 2008. **8**:117.
203. Mergl R, Dogan-Sander E, Willenberg A, Wirkner K, Kratzsch J, Riedel-Heller S, et al. *The effect of depressive symptomatology on the association of vitamin D and sleep.* BMC psychiatry 2021. **21**(1):178.
204. Sharifan P, Khoshakhlagh M, Khorasanchi Z, Darroudi S, Rezaie M, Safarian M, et al. *Efficacy of low-fat milk and yogurt fortified with encapsulated vitamin D(3) on improvement in symptoms of insomnia and quality of life: Evidence from the SUVINA trial.* Food Sci Nutr 2020. **8**(8):4484-90.
205. Bischoff-Ferrari HA, Orav EJ, Abderhalden L, Dawson-Hughes B, Willett WC. *Vitamin D supplementation and musculoskeletal health.* Lancet Diabetes Endocrinol 2019. **7**(2):85.
206. Bolland MJ, Grey A, Avenell A. *Vitamin D supplementation and musculoskeletal health - Authors' reply.* Lancet Diabetes Endocrinol 2019. **7**(2):88-9.
207. Jorde R, Stunes AK, Kubiak J, Joakimsen R, Grimnes G, Thorsby PM, et al. *Effects of vitamin D supplementation on bone turnover markers and other bone-related substances in subjects with vitamin D deficiency.* Bone 2019. **124**:7-13.
208. LeBoff MS, Chou SH, Murata EM, Donlon CM, Cook NR, Mora S, et al. *Effects of Supplemental Vitamin D on Bone Health Outcomes in Women and Men in the VITamin D and OmegA-3 TriaL (VITAL).* J Bone Miner Res 2020. **35**(5):883-93.
209. Crilly RG, Cox L. *A comparison of bone density and bone morphology between patients presenting with hip fractures, spinal fractures or a combination of the two.* BMC Musculoskelet Disord 2013. **14**(1):68.
210. Lips P. *Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications.* Endocr Rev 2001. **22**(4):477-501.
211. Vanderschueren D, Laurent MR, Claessens F, Gielen E, Lagerquist MK, Vandenput L, et al. *Sex steroid actions in male bone.* Endocr Rev 2014. **35**(6):906-60.

Errata

Paper III.

The abbreviation “BMD” has erroneously been defined as “bone mass density” in the abstract (page 1, line 13), in Table 1, Table 3 and in Supplemental Table 1-3. The correct reading is “bone mineral density”.

In the Methods-section, page 2, second column, lines 7-8, the correct age-range of the subjects should read 38-80, not 25-80.

Paper I

Larsen AU, Hopstock LA, Jorde R, Grimnes G.

Vitamin D and sleep in the Tromsø study: Results from a population-based health survey.

Manuscript.

Associations of serum 25-hydroxyvitamin D and self-reported sleep measures in an Arctic population: Insights from the population-based Tromsø Study.

A. U. Larsen[1], L. A. Hopstock[2], R. Jorde[1,3], G. Grimnes[1,3].

[1]Department of Clinical Medicine, UiT The Arctic University of Norway, 9037 Tromsø, Norway.

[2]Department of Community Medicine, UiT The Arctic University of Norway, Tromsø, Norway.

[3]Division of Internal Medicine, University Hospital of North Norway, 9038 Tromsø, Norway.

Corresponding author:

Anette U. Larsen, Department of Clinical Medicine, UiT The Arctic University of Norway, 9037 Tromsø, Norway.

E-mail: anette.uhlving@post.uit.no Phone: +4746824835

Abbreviations[1]

[1] 25(OH)D: 25-hydroxyvitamin D; ISD: Inadequate sleep duration; EDS: Excessive daytime sleepiness; BMI: Body mass index; OSA: Obstructive sleep apnea; HSCL-10: Hopkins Symptoms Check List 10; DAG: Directed acyclic graph; RCT: Randomized controlled trial.

Abstract

Objective: To investigate the relationship between serum 25-hydroxyvitamin D (s-25(OH)D) and self-reported sleep measures in an Arctic population living in Northern Norway at 69°N.
Methods: The present study used cross-sectional data collected in the population based Tromsø Study: Tromsø7 (2015-2016), in which 21,083 Tromsø inhabitants aged ≥ 40 years participated. In total, 20,438 participants were included, after having excluded respondents with missing s-25(OH)D measurement (n=161) and without data on one at least one of the following sleep measures: sleep duration, insomnia, or daytime sleepiness (n=490). Vitamin D status was based on s-25(OH)D (assessed using LC-MS/MS) and categorized as deficient (< 30 nmol/L), insufficient (30-49.9 nmol/L), sufficient (50-75 nmol/L) and high (> 75 nmol/L). Sleep duration was categorized as inadequate (ISD) if < 7 or ≥ 9 hours. Linear and logistic regression were used to estimate associations between s-25(OH)D and sleep measures, calculating unstandardized β-values and odds ratios [95% confidence intervals]. The regression analyses were adjusted for season, age, BMI, lifestyle factors and relevant comorbidities.
Results: In men, there were no significant associations between s-25(OH)D and self-reported sleep measures in the fully adjusted models. In women, s-25(OH)D was positively associated with sleep duration (adjusted β = 0.8 minutes /10 nmol/L, p=.013) and the insufficient s-25(OH)D group had higher odds of ISD compared to the sufficient s-25(OH)D group, after having adjusted for confounding factors (1.16 [1.03, 1.32] p=.017). Women with high s-25(OH)D level had higher odds of insomnia compared to women in the sufficient s-25(OH)D group (1.16 [1.01-1.33], p=.036). There were no associations with daytime sleepiness.
Conclusions: A tenuous association was found between s-25(OH)D and self-reported sleep measures in this Arctic population. The results conflicted according to sex and sleep measure, and most associations were lost when adjusted for confounding factors.

Keywords: Sleep duration; Insomnia; Excessive daytime sleepiness; Vitamin D; 25(OH)D.

1. Introduction

Sleep is an essential component of our daily routine and function as human beings. Adequate duration, timing, regularity, and quality of sleep is required to maintain critical brain functions and influences many systemic processes (1). Sleep disturbances has been reported to increase the risk of both mental and somatic diseases, including cardiovascular and cerebrovascular diseases, obesity, diabetes, cancer, and depression (2). Although sleep-demands show great intra- and inter-individual variability depending on age, sex, and environmental factors (1), adults are in general recommended 7-9 hours of sleep on a daily basis (1, 3). The proportion of adults not reaching this goal is worryingly high (1). Both environmental and societal factors, such as family responsibilities, nonstandard working hours (4), and an increased use of electronic devices and screen time (5), may explain some of the discrepancies between the amount of sleep recommended and the amount of sleep obtained. However, additional factors remain to be explored.

Vitamin D is one such factor. Besides its crucial role in maintenance of calcium and phosphorus homeostasis, vitamin D has been increasingly recognized as a steroid prohormone that exerts a variety of functions through its vitamin D receptor (VDR) (6). In the last decade, emerging evidence have spoken in favour of an association between vitamin D and sleep health (7), as vitamin D deficiency is prevalent in populations with sleep complaints (8). Considering that its metabolites are capable of crossing the blood brain barrier (9), this link is biologically plausible. The sleep-wake cycle is regulated by the circadian-clock and hormones produced by the hypothalamus (10). A number of environmental inputs modulate its functions (10), and vitamin D may possibly play an independent role. The VDR has been found in areas of the human brain known to be involved in the regulation of sleep, along with key enzymes required for vitamin D metabolism (10, 11). Biological mechanisms through which vitamin D may exert its effects on sleep have been suggested, including regulation of circadian clock genes, alterations of melatonin synthesis and through its role as an immune modulator (12, 13).

In observational studies, vitamin D has been associated with less difficulty maintaining sleep (14). Higher concentrations of serum 25-hydroxyvitamin D (s-25(OH)D), the main circulating vitamin D metabolite, have been associated with longer and earlier night sleep (15). Moreover, vitamin D deficiency has been associated with short sleep duration (16-23), poor self-reported sleep quality (24, 25), and an increased risk of daytime sleepiness (26, 27). However, neither of these studies were done in populations living under Arctic conditions, in which a worryingly high prevalence of both insufficient sleep duration and insomnia have been reported (28).

Thus, the aim of the present study was to examine the association between s-25(OH)D and self-reported sleep measures in a general population living in the Arctic area of Northern Norway, with significant variations in daylength and sunlight exposure throughout the year (29).

2. Materials and Methods

2.1 The Tromsø Study: Population and study design

The Tromsø Study is a longitudinal, population-based health study conducted in the municipality of Tromsø in Northern Norway (30). Tromsø is geographically situated at 69° North, with extreme variations in daylight throughout the year, including two months of polar night during winter and two months of midnight sun during summer. The Tromsø Study consists of seven repeated cross-sectional surveys conducted between 1974 and 2016 (Tromsø 1–Tromsø 7), to which total birth cohorts and random samples have been invited. In Tromsø 7 (2015-2016), all inhabitants aged 40 years and above (N = 32,591) were invited, of which 21,083 women and men aged 40-99 years participated (attendance rate 65%). Data collection included questionnaires, biological sampling, and clinical examinations, as described in detail below.

2.2 Variables and measurements

2.2.1 Vitamin D and anthropometric measurements

Serum sampling was performed non-fasting, and s-25(OH)D was measured using the gold standard LC-MS/MS method at the Department of Clinical Biochemistry, University Hospital of North Norway. The analysis was accredited by the Norwegian Accreditation Authority and the laboratory participates in the international quality surveillance programme the vitamin D external quality assurance scheme (DEQAS) to ensure the analytical reliability of the 25(OH)D assays. Subgroup classification of vitamin D status was done according to s-25(OH)D level and performed in accordance with recent recommendations (31, 32). Thus, vitamin D deficiency was defined as s-25(OH)D values < 30 nmol/L, and vitamin D insufficiency as values from 30 nmol/L to 50 nmol/L. Values from 50 through 75 nmol/L were defined as sufficient, whereas values > 75 nmol/L were categorized as high.

Height and weight were measured wearing light clothing and without shoes. Body mass index (BMI) was calculated as weight (kilograms) divided by height squared ($meter^2$). All measurements were performed by trained technicians.

2.2.3 Self-reported sleep measures

Each participant completed an extensive questionnaire including questions about habitual sleep traits to assess self-reported sleep measures, including sleep duration, symptoms of insomnia, and daytime sleepiness. The questionnaire was completed online before the study visit or at the examination site, aided by the study personnel as needed. The questionnaire items used to assess self-reported sleep measures are summarized in Supplemental Figure 1.

Sleep duration was calculated as time in bed (bedtime subtracted from rise time) minus sleep onset latency. Sleep duration was further categorized into normal (7-9 hours) and inadequate sleep duration (ISD) (< 7 h or ≥ 9 hours), in accordance with age-specific recommendations (3).

Insomnia was assessed using a slightly modified version of the Bergen Insomnia Scale (BIS) (33), adjusted to adhere with recent guidelines on diagnosing insomnia, including the Diagnostic and Statistical Manual for Mental Disorders, Fifth Edition (DSM-5) (34) and International Classification of Sleep Disorders, Third Edition (ICSD-3) (35). Insomnia was defined in accordance with *Sivertsen et al.* (28) and categorized as present if the participants reported: 1) at least one of three nocturnal symptoms (prolonged sleep onset latency, difficulties maintaining sleep and/or early morning awakening) ≥ 3 nights/week, and 2) one or both of two daytime symptoms (daytime sleepiness/tiredness and/or dissatisfaction with sleep) ≥ 3 days/week, and 3) a duration of sleep problems for ≥ 3 months.

Daytime sleepiness was assessed using the Epworth Sleepiness Scale (ESS) (36). The ESS is a 4-point Likert-scale questionnaire composed of 8 items, in which the participant marks the probability of dozing while engaging certain daily activities. The score for each item varies from 0 (no chance of dozing) to 3 (high chance of dozing). The total score ranges from 0-24 points. Excessive daytime sleepiness (EDS) was defined as an ESS score of > 10, in accordance with *Johns et al.* (37).

2.2.4 Covariates

Covariates were selected based on available empirical evidence summarized in a directed acyclic graph (DAG) (Supplemental Figure 2). The following information was obtained: BMI, smoking status (current *vs*. not current), alcohol consumption (gram/day), leisure time physical activity (sedentary/low *vs*. moderate/vigorous), marital status (living with spouse yes/no), self-perceived economy (difficult *vs*. average or good), shift work (yes/no), obstructive sleep apnoea (OSA) (yes/no), cardiovascular disease (myocardial infarction, stroke, atrial fibrillation, angina and/or heart failure), cancer (current *vs*. not current) and/or presence of constant or constantly reoccurring chronic

pain during the last 3 months or more (yes/no), and afflictions of night sweats (not at all/not so much *vs*. sometimes/definitely), the latter regarding women only. Symptoms of psychological distress were assessed using the Hopkins Symptoms Check List 10 (HSCL-10), with a HSCL-10-score cut-off of ≥ 1.85 (38).

2.3 Statistical analyses

Normal distribution was evaluated by visual inspection of histograms and by determination of kurtosis and skewness. Continuous data was presented as means and standard deviations. Proportions were provided as number of participants and percentages. As previous studies have reported sex-differences in sleep (39), all analyses were performed sex-stratified.

The prevalence of each categorical sleep measure (i.e., ISD, insomnia and EDS) was compared between men and women using the Pearson's Chi-square test. Differences across s-25(OH)D groups were tested using the Pearson's Chi-square test for categorical variables and one-way analysis of variance for continuous variables. Mean s-25(OH)D level was compared between men and women and within each categorical sleep measure using the Student's *t*-test.

To identify a valid adjustment set for the regression analyses, DAGs were constructed using the DAGitty v3.0 software. The final DAG (Supplemental Figure 1) was selected through evaluation of multiple DAGs combined with available empirical evidence. Multivariate binary logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for each categorical sleep measure. Two separate analyses were performed for each categorical sleep measure using s-25(OH)D as 1) a continuous predictor variable and 2) a categorical predictor variable. When used as a categorical predictor variable, each s-25(OH)D group was dichotomized and added to the regression model as an independent variable, excluding the vitamin D sufficient group, which was used as the reference group. In crude analyses, the ORs were adjusted for month of serum sampling by adding each month as an independent, dummy variable (Model 1). In the final model (Model 2), relevant confounding variables were added to the regression, including month of serum sampling, age, BMI, smoking, alcohol intake, leisure time physical activity, living with a spouse or partner, self-perceived economy, shift work, OSA, psychological distress and other relevant comorbidities (including cardiovascular disease, cancer and/or chronic pain during the last 3 months or more). In addition, the regression models in women were also adjusted for afflictions of night sweat.

Multiple linear regression was used to model the relationship between the continuous sleep measures (i.e., sleep duration and ESS-score). Using the same models as described for the logistic regression

analyses, two separate analyses were performed for each continuous sleep measure (i.e., sleep duration and ESS-score) using s-25(OH)D as 1) a continuous predictor variable and 2) a categorical predictor variable.

Relevant interactions, including [s-25(OH)D × sex], [s-25(OH)D × age], [s-25(OH)D × BMI], and [s-25(OH)D × depression] were tested by adding the multiplicative term of the two variables in crude analyses. There were no significant interactions with s-25(OH)D, except for sex when applied in the regression model with insomnia as the dependent variable (p=.012).

The statistical analyses were performed using SPSS software version 27.0 (IBM Corp, Chicago, IL). All tests were performed two-sided, and p-values < 0.05 were considered statistically significant.

2.4 Ethics

All of the participants provided written informed consent prior to participating in the Tromsø Study. The present study was approved by the Norwegian Committee for Medical and Health Research Ethics (REC North ref. 2020/7614).

3. Results

3.1 Sample characteristics

Sample characteristics of the study population are shown in Table 1. In the present study, the sample consisted of 20,438 individuals after having excluded participants missing s-25(OH)D measurement (n=161) and/or without data on at least one sleep measure (n=490). The participant inclusion is shown in Figure 1. The 645 excluded participants were significantly older and more likely to report comorbidities (including cardiovascular disease, a current cancer diagnosis and/or chronic pain during the last 3 months or more). The mean alcohol intake was significantly lower among the excluded participants, and they were less likely to report moderate or vigorous leisure time physical activity, living with a spouse or partner, and having shift work, compared to the included participants. The characteristics of the excluded participants are also summarized in Table 1.

3.2 Self-reported sleep measures

Participants with missing data on a specific sleep measure were only excluded from the analyses for which data were missing (sleep duration (n=1,241), insomnia (n=901), daytime sleepiness (n=1,128)).

ISD was the most prevalent categorical sleep measure in both sexes (Table 2), and the prevalence was significantly higher in men compared to women (53.9% vs. 45.8%, p <.001). Participants with ISD had significantly lower mean s-25(OH)D compared to those reporting a normal sleep duration, in both men (59.2 ± 20.8 nmol/L vs. 62.4 ± 21.4 nmol/L, p <.001) and women (64.6 ± 22.5 nmol/L vs. 66.9 ± 22.1 nmol/L, p <.001). In both sexes, a linear trend was observed across s-25(OH)D groups, with a falling prevalence of ISD from the group with the lowest to the highest level of s-25(OH)D (Table 2, Figure 2). Using logistic regression adjusted for month of serum sampling (Model 1), the odds of ISD was significantly lower with higher s-25(OH)D in both men and women when the continuous s-25(OH)D variable was applied (Table 3). Using the categorical variable, the odds of ISD was significantly higher in the deficient and insufficient groups compared to the sufficient s-25(OH)D group in both sexes (Model 1, Table 4). In men, the high-level group had significantly lower odds of ISD compared to the sufficient group, when adjusted for month of serum sampling (Table 4, Model 1). With additional adjustment (Model 2), all estimates were attenuated and remained statistically significant only in men when the continuous s-25(OH)D variable was used (Table 3), and only in women with insufficiency when the categorical s-25(OH)D variable was used (Table 4). Using linear regression adjusted for month of serum sampling (Model 1), s-25(OH)D was positively associated with sleep duration in both men and women using both the continuous (unstandardized β for every 10 nmol/L increase: 2.3 minutes, p <.001, and 1.7 minutes, p <.001, in men and women respectively) and the categorical variable (Model 1, Table 5). With additional adjustment for potential confounders (Model 2), the association with the continuous s-25(OH)D variable remained significant only in women (unstandardized β for every 10 nmol/L increase: 0.6 minutes, p =.092 and 0.8 minutes, p =.013, in men and women respectively). When using the categorical s-25(OH)D variable, both men and women with s-25(OH)D insufficiency had significantly shorter sleep duration compared to s-25(OH)D sufficient group in the fully adjusted analysis (Model 2, Table 5).

The prevalence of insomnia was significantly lower in men compared to women (15.2% vs. 24.7%, p <.001). Men with insomnia had significantly lower mean s-25(OH)D compared to men without (59.8 ± 21.8 nmol/L vs 61.0 ± 21.0 nmol/L, p =.047). In contrast, women with insomnia had significantly higher mean s-25(OH)D compared to women without (67.9 ± 23.2 nmol/L vs 65.5 ± 22.0 nmol/L, p <.001). In men, the prevalence of insomnia did not differ significantly across s-25(OH)D groups (Table 2, Figure 3) and there were no significant associations between s-25(OH)D and insomnia in men, regardless of the model used (Table 3-4). In women, the prevalence of insomnia was significantly higher in the high-level group (Table 2, Figure 3). Adjusted for month of serum

sampling (Model 1), the odds of insomnia was significantly higher with increasing s-25(OH)D (Table 3) and compared to women in the sufficient s-25(OH)D group, the odds of insomnia was significantly higher among women in the high-level group (Table 4). The association between s-25(OH)D and insomnia remained statistically significant with additional adjustments (Model 2), both when using the continuous (Table 3) and the categorical s-25(OH)D variable (Table 4).

The prevalence of EDS was the same in men and women (Table 2). Participants with EDS had significantly lower mean s-25(OH)D as compared to the participants without EDS, both in men (59.2 ± 21.2 nmol/L vs. 60.9 ± 21.1 nmol/L, p =.020) and in women (66.2 ± 22.3 nmol/L vs. 64.5 ± 64.5 nmol/L, p =.017). A linear trend was observed across s-25(OH)D groups, with a falling prevalence of EDS from the group with the lowest to the highest level of s-25(OH)D, which was more prominent in women than in men (Table 2, Figure 4). Using logistic regression adjusted for month of serum sampling (Model 1), the odds of EDS was significantly lower with higher s-25(OH)D in both men and women when the continuous s-25(OH)D variable was applied (Table 3). Using the categorical variable, the association with EDS was significant only in men with high s-25(OH)D levels, in which the odds of EDS was lower compared to the sufficient s-25(OH)D group (Model 1, Table 4). With additional adjustment (Model 2), all estimates were attenuated and no longer statistically significant, regardless of the s-25(OH)D variable used (Table 4). Modelled using linear regression, there were no significant associations between s-25(OH)D as a continuous variable and ESS-score in men, neither adjusted for month of serum sampling (unstandardized β for every 10 nmol/L increase: -0.03 (-0.06, 0.01), p = .137), nor in the fully adjusted analyses (0.02 (-0.02, 0.06), p = .339). This result persisted when the categorical variable was applied (Table 5). In women, there was an inverse association between higher s-25(OH)D (continuous) and lower ESS-scores when adjusted for month of serum sampling (unstandardized β for every 10 nmol/L increase: -0.09 (-0.12, -0.05), p <.001). With additional adjustment (Model 2), the association was attenuated and no longer significant using the continuous s-25(OH)D variable (0.01 (-0.03, 0.04), p = .777). When using the categorical s-25(OH)D variable, women in the high-level group had significantly lower ESS-scores compared to women in the s-25(OH)D sufficient group (Model 2, Table 5).

Sensitivity analyses were performed stratifying the study population according to the season of participation, including a summer group (April through September), and a winter group (October through March). With full adjustment (Model 2), the findings in the winter group were similar as described for the main analyses in both sexes, except a significantly increased odds of ISD in the s-25(OH)D deficient and insufficient groups in men, and the association with insomnia in women was

no longer significant (Supplemental Table 1-3). In the summer group, there were no significant associations with s-25(OH)D when the fully adjusted models were applied (Supplemental Table 4-6).

4. Discussion

To our knowledge, this is the first study to examine the association between s-25(OH)D and self-reported sleep measures in an Arctic population. In this cross-sectional analysis, a tenuous association was found, but the results conflicted according to sex, s-25(OH)D group and sleep measure when adjusted for relevant confounding factors.

Some of our findings conflicted with the results of a recent meta-analysis by *Gao et al.* (7). Based on the findings of nine observational studies (22-24, 27, 40-43) it was concluded that vitamin D deficiency was associated with shorter sleep duration and an increased risk of poor sleep quality and daytime sleepiness. However, comparison of our findings with previous literature is complicated by the substantial variation in methodology, definition of outcomes and covariate adjustment, as will be discussed in the following.

A positive association between 25(OH)D and sleep duration has been reported in several recent studies, in both men and women (15, 16, 18-23). Others have reported null findings (44, 45). In the present study, we found conflicting results regarding the association between s-25(OH)D and self-reported sleep duration. In both sexes, s-25(OH)D was positively associated with sleep duration when adjusted for month of serum sampling, but with additional adjustment the association was only significant in women. In support of this association, the fully adjusted odds of ISD was significantly increased in women with s-25(OH)D insufficiency compared to women with sufficient s-25(OH)D levels (defined here from 30 to 50 nmol/L and 50 to 75 nmol/L, respectively). In contrast, when s-25(OH)D was used as a continuous variable, the association with ISD was only significant in men, in the fully adjusted model. Interestingly, *Bertisch et al.* (17) found a significant association between s-25(OH)D and objectively measured sleep duration but found no association with self-reported sleep duration. Whether this could have explained some of the discrepancies in our study is unclear, as the analyses by *Bertisch et al.* (17) were not stratified by sex.

Conflicting with the conclusion regarding sleep quality in the meta-analysis by *Gao et al.* (7), we did not find a significantly increased risk of insomnia with lower s-25(OH)D levels in the present study. There are some important aspects that ought to be considered when comparing these results; *Gao et al.* (7) included only four studies with more than 1000 participants (22, 24, 27, 46). Only two of these studies included data on sleep quality (24, 46) and neither of these studies matched the present

study population of middle-aged and older (± 40 years) men and women. Surprisingly, the present study showed a lower risk of insomnia in women with sufficient as compared to high s-25(OH)D levels (defined here as 50-75 nmol/L and > 75 nmol/L, respectively). Our study is not the first observational study to have linked high s-25(OH)D to unfavourable self-reported sleep measures; In a cross-sectional analysis by *Shiue et al.* (45) individuals with higher s-25(OH)D levels were found more likely to report sleep complaints. Moreover, in a double-blind, placebo controlled RCT with vitamin D in 218 postmenopausal women, *Mason et al.* (47) found that women who became vitamin D replete (≥32 ng/ml or 80 nmol/L) reported a deterioration in sleep quality (48). In contrast, two recent double-blind placebo controlled RCTs (49, 50) found a positive effect of vitamin D to improve sleep quality. The mean end-of-study 25(OH)D level in the vitamin D groups of these studies were 37.7 ng/ml (~94.2 nmol/L) (50) and 22 ng/ml (~55 nmol/L). However, both studies had associated limitations such as small sample size (< 100 participants) and not using the gold standard method LC-MS/MS to assess 25(OH)D. Also, analyses to explore potential subgroup effects according to achieved 25(OH)D level were not performed.

Due to the considerable attention in mainstream media devoted to extra-skeletal effects of vitamin D, it cannot be excluded that an increased odds of insomnia with higher s-25(OH)D might represent reversed causation. It could be hypothesized that women with insomnia may have been more likely to start taking a vitamin D supplement in an attempt to improve their sleep problems. This was partially seen in our study, as the proportion reporting to use a vitamin D supplement was significantly higher among individuals with insomnia compared to those without, in both sexes. However, as there were more women than men with insomnia, it cannot be excluded that the association was only significant in women simply because of the higher prevalence, increasing the probability of finding a statistically significant result. Nevertheless, the analyses in the present study were not adjusted for vitamin D supplement use, as the aim was to estimate the association between self-reported sleep measures and s-25(OH)D from all sources.

The present study found no associations between s-25(OH)D and daytime sleepiness. This finding contrasted with previous studies suggesting an inverse association between vitamin D and sleepiness (26, 27). Yet, with appropriate covariate adjustment, others have reported null findings (15, 17).

The present study has methodological limitations. First, all sleep measures were assessed using self-report, which has been reported to show less consistent findings than studies using objective sleep measures (15). Yet, even objective sleep measurements vary depending on the methods used (i.e., whether obtained using a gold standard polysomnography or based on more approximate methods,

such as actigraphy) (51). Objective sleep recordings are typically performed over a short period of time (usually 1-7 days). Thus, such measurements represents a "snapshot" of an individual's sleep health status, whereas the focus of the present study was on habitual sleep patterns. Second, the prevalence of vitamin D deficiency (s-25(OH)D < 30 nmol/L) was low the present study, compromising the power to identify significant associations (especially for subgroup analyses). Third, the study population consisted of adults aged 40 years and older, living in an Arctic area of Northern Norway. Extrapolation of the results to younger age-groups or to populations living under different conditions might not be appropriate. Fourth, DAGs were used to identify relevant confounding factors. However, an important premise of DAGs lies explicitly in the name, emphasizing the existence of an acyclic relationship between the exposure and the outcome. Many of the covariates treated as confounding factors in the present study could have been related to s-25(OH)D level in an indirect, or even bidirectional manner, for instance through variations in diet, BMI, and physical activity. Finally, a cross-sectional study design such as in the present study cannot infer causal relationships. The only appropriate way to determine whether the association between s-25(OH)D and sleep health reflects a true causal relationship, rather than confounding or reverse causation, is through well-designed RCTs (52).

The present study also has its strengths, such as the large sample size of more than 20 000 participants and the high attendance rate (65%), both improving generalizability of the results. The categorical sleep measures were adequately prevalent with regards to the study's aim of detecting significant associations between s-25(OH)D and self-reported sleep measures.

5. Conclusion

In conclusion, a tenuous association was found between s-25(OH)D and self-reported sleep measures in this Arctic population, but with conflicting results according to sex, s-25(OH)D group and sleep measure studied. Statistical significance was lost for most of the comparisons when adjusted for confounding factors. Given the cross-sectional design of the study, causal inferences regarding the relationship between vitamin D and self-reported sleep measures could not be drawn, and the effect of vitamin D supplementation on sleep ought to be settled through RCTs.

Acknowledgements

The authors would like to thank all the participants and the staff in the Tromsø Study. The authors also gratefully acknowledge the contributions made by Børge Sivertsen providing the syntax for the definition of the sleep duration and insomnia variables, by Sarah Anne Cook providing the syntax for

the definition of the alcohol intake variable, and by Tom Wilsgaard providing valuable insights regarding the statistical analyses.

Funding

This work was supported by grants from the North Norway Regional Health Authorities (grant number SFP1277-16) and UiT The Arctic University of Norway to perform analyses of s-25(OH)D. AUL received funding through the PhD Program at UiT The Arctic University of Norway. The publication charges for this article were funded through UiT The Arctic University of Norway's Publishing Agreements. The funding body did not have a role in the design of the study, data collection, analysis, interpretation of data or writing of the manuscript.

Declarations of interest

The authors have no competing interests to declare.

References

1. Watson NF, Badr MS, Belenky G, et al. Joint Consensus Statement of the American Academy of Sleep Medicine and Sleep Research Society on the Recommended Amount of Sleep for a Healthy Adult: Methodology and Discussion. J Clin Sleep Med 2015;11(8):931-52. https://doi.org/10.5664/jcsm.4758.
2. Cappuccio FP, D'Elia L, Strazzullo P, et al. Sleep duration and all-cause mortality: a systematic review and meta-analysis of prospective studies. Sleep 2010;33(5):585-92. https://doi.org/10.1093/sleep/33.5.585.
3. Hirshkowitz M, Whiton K, Albert SM, et al. National Sleep Foundation's updated sleep duration recommendations: final report. Sleep Health 2015;1(4):233-43. https://doi.org/10.1016/j.sleh.2015.10.004.
4. Pallesen S, Sivertsen B, Nordhus IH, et al. A 10-year trend of insomnia prevalence in the adult Norwegian population. Sleep Med 2014;15(2):173-9. https://doi.org/10.1016/j.sleep.2013.10.009.
5. Hysing M, Pallesen S, Stormark KM, et al. Sleep and use of electronic devices in adolescence: results from a large population-based study. BMJ Open 2015;5(1):e006748. https://doi.org/10.1136/bmjopen-2014-006748.
6. Scragg R, Sluyter JD. Is There Proof of Extraskeletal Benefits From Vitamin D Supplementation From Recent Mega Trials of Vitamin D? JBMR Plus 2021;5(1):e10459. https://doi.org/10.1002/jbm4.10459.
7. Gao Q, Kou T, Zhuang B, et al. The Association between Vitamin D Deficiency and Sleep Disorders: A Systematic Review and Meta-Analysis. Nutrients 2018;10(10):1395. https://doi.org/10.3390/nu10101395.
8. Gominak SC, Stumpf WE. The world epidemic of sleep disorders is linked to vitamin D deficiency. Med Hypotheses 2012;79(2):132-5. https://doi.org/ 10.1016/j.mehy.2012.03.031.
9. Pardridge WM, Sakiyama R, Coty WA. Restricted transport of vitamin D and A derivatives through the rat blood-brain barrier. J Neurochem 1985;44(4):1138-41. https://doi.org/10.1111/j.1471-4159.1985.tb08735.x.
10. Saper CB, Cano G, Scammell TE. Homeostatic, circadian, and emotional regulation of sleep. J Comp Neurol 2005;493(1):92-8. https://doi.org/10.1002/cne.20770.
11. Eyles DW, Smith S, Kinobe R, et al. Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain. J Chem Neuroanat 2005;29(1):21-30. https://doi.org/10.1016/j.jchemneu.2004.08.006.
12. Romano F, Muscogiuri G, Di Benedetto E, et al. Vitamin D and Sleep Regulation: Is there a Role for Vitamin D? Curr Pharm Des 2020;26(21):2492-6. https://doi.org/10.2174/1381612826666200310145935.
13. Calton EK, Keane KN, Newsholme P, et al. The Impact of Vitamin D Levels on Inflammatory Status: A Systematic Review of Immune Cell Studies. PloS one 2015;10(11):e0141770. https://doi.org/ 10.1371/journal.pone.0141770.
14. Grandner MA, Jackson N, Gerstner JR, et al. Sleep symptoms associated with intake of specific dietary nutrients. J Sleep Res 2014;23(1):22-34. https://doi.org/10.1111/jsr.12084.
15. Dogan-Sander E, Willenberg A, Batmaz İ, et al. Association of serum 25-hydroxyvitamin D concentrations with sleep phenotypes in a German community sample. PloS One 2019;14(7):e0219318. https://doi.org/10.1371/journal.pone.0219318.
16. Beydoun, M. A., Ng, A. E., Fanelli-Kuczmarski, et al. Vitamin D status and its longitudinal association with changes in patterns of sleep among middle-aged urban adults. J Affect Disord 2021;282:858-868. https://doi.org/10.1016/j.jad.2020.12.145.

17. Bertisch SM, Sillau S, de Boer IH, et al. 25-Hydroxyvitamin D Concentration and Sleep Duration and Continuity: Multi-Ethnic Study of Atherosclerosis. Sleep. 2015;38(8):1305-11. https://doi.org/10.5665/sleep.4914.
18. Doo M. The Association between Sleep Duration and 25-Hydroxyvitamin D Concentration with Obesity in an Elderly Korean Population: A Cross-Sectional Study. Nutrients 2018 10(5)575. https://doi.org/10.3390/nu10050575Doo 2018
19. Kim JH, Chang JH, Kim DY, et al. Association between self-reported sleep duration and serum vitamin D level in elderly Korean adults. J Am Geriatr Soc 2014;62(12):2327-32. https://doi.org/10.1111/jgs.13148.
20. Kim SY, Lee MH, Lim WJ, et al. Associations of 25-Hydroxyvitamin D Levels and Arthritis with Sleep Duration: The Korean National Health and Nutrition Examination Survey 2008-2014. Nat Sci Sleep 2020;12:883-94. https://doi.org/10.2147/NSS.S275464.
21. Liu X, Ke L, Ho J, et al. Sleep duration is associated with vitamin D deficiency in older women living in Macao, China: A pilot cross-sectional study. PloS One 2020;15(3):e0229642. https://doi.org/10.1371/journal.pone.0229642.
22. Massa J, Stone KL, Wei EK, et al. Vitamin D and actigraphic sleep outcomes in older community-dwelling men: the MrOS sleep study. Sleep 2015;38(2):251-7. https://doi.org/10.5665/sleep.4408.
23. Piovezan RD, Hirotsu C, Feres MC, et al. Obstructive sleep apnea and objective short sleep duration are independently associated with the risk of serum vitamin D deficiency. PloS One 2017;12(7):e0180901. https://doi.org/10.1371/journal.pone.0180901.
24. Jung YS, Chae CH, Kim YO, et al. The relationship between serum vitamin D levels and sleep quality in fixed day indoor field workers in the electronics manufacturing industry in Korea. Ann Occup Environ Med 2017;29:25. https://doi.org/10.1186/s40557-017-0187-7.
25. Nakamura K, Hui SP, Ukawa S, Okada E, Nakagawa T, Okabe H, et al. Serum 25-hydroxyvitamin D(3) levels and poor sleep quality in a Japanese population: the DOSANCO Health Study. Sleep Med 2019;57:135-40. https://doi.org/10.1016/j.sleep.2019.01.046.
26. McCarty DE, Reddy A, Keigley Q, et al. Vitamin D, race, and excessive daytime sleepiness. J Clin Sleep Med 2012;8(6):693-7. https://doi.org/10.5664/jcsm.2266.
27. Beydoun MA, Gamaldo AA, Canas JA, et al. Serum nutritional biomarkers and their associations with sleep among US adults in recent national surveys. PloS One 2014;9(8):e103490. https://doi.org/10.1371/journal.pone.0103490.
28. Sivertsen B, Pallesen S, Friborg O, et al. Sleep patterns and insomnia in a large population-based study of middle-aged and older adults: The Tromsø study 2015-2016. J Sleep Res 2020;30(1):e13095. https://doi.org/10.1111/jsr.13095.
29. Engelsen O, Brustad M, Aksnes L, et al. Daily Duration of Vitamin D Synthesis in Human Skin with Relation to Latitude, Total Ozone, Altitude, Ground Cover, Aerosols and Cloud Thickness. Photochem Photobiol 2005;81(6):1287-90. https://doi.org/10.1562/2004-11-19-RN-375.
30. Jacobsen BK, Eggen AE, Mathiesen EB, et al. Cohort profile: the Tromso Study. Int J Epidemiol 2012;41(4):961-7. https://doi.org/10.1093/ije/dyr049.
31. Amrein K, Scherkl M, Hoffmann M, et al. Vitamin D deficiency 2.0: an update on the current status worldwide. Eur J Clin Nutr 2020;74(11):1498-513. https://doi.org/10.1038/s41430-020-0558-y.
32. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011;96(7):1911-30. https://doi.org/10.1210/jc.2011-0385.
33. Pallesen S, Bjorvatn B, Nordhus IH, et al. A new scale for measuring insomnia: the Bergen Insomnia Scale. Percept Mot Skills 2008;107(3):691-706. https://doi.org/10.2466/pms.107.3.691-706.

34. American Psychiatric Association. Diagnostic and statistical manual of mental disorders (5th ed). Washington: American Psychiatric Association Publishing: 2013.
35. American Academy of Sleep Medicin e. International Classification of Sleep Disorders, 3rd ed. (ICSD-3). Darien, IL: American Academy of Sleep Medicine 2014.
36. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 1991;14(6):540-5. https://doi.org/10.1093/sleep/14.6.540.
37. Johns MW. The official website of the Epworth Sleepiness Scale (ESS) and the Epworth Sleepiness Scale for Children and Adolecents (ESS-CHADS). 2016: Retrieved on Feb 3rd 2021 from http://www.epworthsleepinessscale.com/.
38. Strand BH, Dalgard OS, Tambs K, et al. Measuring the mental health status of the Norwegian population: a comparison of the instruments SCL-25, SCL-10, SCL-5 and MHI-5 (SF-36). Nord J Psychiatry 2003;57(2):113-8. https://doi.org/10.1080/08039480310000932.
39. Mong JA, Cusmano DM. Sex differences in sleep: impact of biological sex and sex steroids. Philos Trans R Soc Lond B Biol Sci 2016;371(1688):20150110. https://doi.org/10.1098/rstb.2015.0110.
40. Carlander B, Puech-Cathala AM, Jaussent I, et al. Low vitamin D in narcolepsy with cataplexy. PloS one 2011;6(5):e20433. https://doi.org/10.1371/journal.pone.0020433.
41. Dauvilliers Y, Evangelista E, Lopez R, et al. Vitamin D deficiency in type 1 narcolepsy: a reappraisal. Sleep Med. 2017;29:1-6. https://doi.org/10.1016/j.sleep.2016.05.008.
42. Ekinci RMK, Balci S, Serbes M, et al. Decreased serum vitamin B(12) and vitamin D levels affect sleep quality in children with familial Mediterranean fever. Rheumatol Int 2018;38(1):83-7. https://doi.org/10.1007/s00296-017-3883-2.
43. Gunduz S, Kosger H, Aldemir S, et al. Sleep deprivation in the last trimester of pregnancy and inadequate vitamin D: Is there a relationship? J Chin Med Assoc. 2016;79(1):34-8. https://doi.org/10.1016/j.jcma.2015.06.017.
44. Choi JH, Lee B, Lee JY, et al. Relationship between Sleep Duration, Sun Exposure, and Serum 25-Hydroxyvitamin D Status: A Cross-sectional Study. Sci Rep 2020;10(1):4168. https://doi.org/10.1038/s41598-020-61061-8.
45. Shiue I. Low vitamin D levels in adults with longer time to fall asleep: US NHANES, 2005-2006. Int J Cardiol 2013;168(5):5074-5. https://doi.org/ 10.1016/j.ijcard.2013.07.195.
46. Cheng TS, Loy SL, Cheung YB, et al. Plasma Vitamin D Deficiency Is Associated With Poor Sleep Quality and Night-Time Eating at Mid-Pregnancy in Singapore. Nutrients 2017;9(4)340. https://doi.org/10.3390/nu9040340.
47. Mason C, de Dieu Tapsoba J, Duggan C, et al. Repletion of vitamin D associated with deterioration of sleep quality among postmenopausal women. Prev Med 2016;93:166-70. https://doi.org/10.1016/j.ypmed.2016.09.035.
48. Buysse DJ, Reynolds 3rd CF, Monk TH, et al. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res 1989;28(2):193-213. https://doi.org/10.1016/0165-1781(89)90047-4.
49. Majid MS, Ahmad HS, Bizhan H, Hosein HZM, Mohammad A. The effect of vitamin D supplement on the score and quality of sleep in 20-50 year-old people with sleep disorders compared with control group. Nutr Neurosci 2018;21(7):511-9. https://doi.org/ 10.1080/1028415X.2017.1317395.
50. Ghaderi A, Banafshe HR, Motmaen M, Rasouli-Azad M, Bahmani F, Asemi Z. Clinical trial of the effects of vitamin D supplementation on psychological symptoms and metabolic profiles in maintenance methadone treatment patients. Prog Neuropsychopharmacol Biol Psychiatry 2017;79(Pt B):84-9. https://doi.org/10.1016/j.pnpbp.2017.06.016.
51. Withrow D, Roth T, Koshorek G, et al. Relation between ambulatory actigraphy and laboratory polysomnography in insomnia practice and research. J Sleep Res 2019;28(4):e12854. https://doi.org/10.1111/jsr.12854.

52. Jorde R. RCTS are the only appropriate way to demonstrate the role of vitamin D in health. J Steroid Biochem Mol Biol 2018;177:10-4. https://doi.org/10.1016/j.jsbmb.2017.05.004.

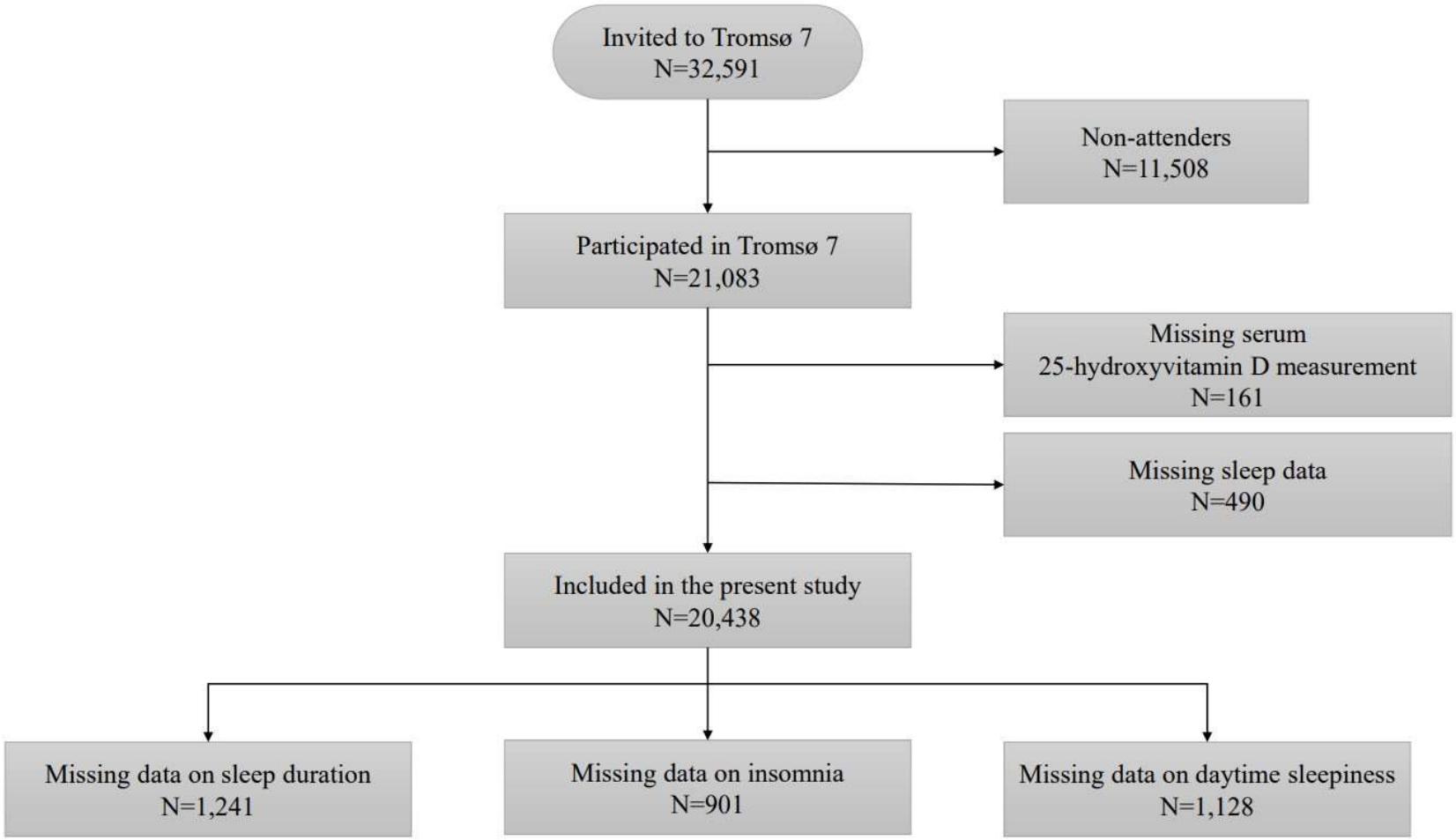


Figure 1. Flow diagram of participant inclusion.

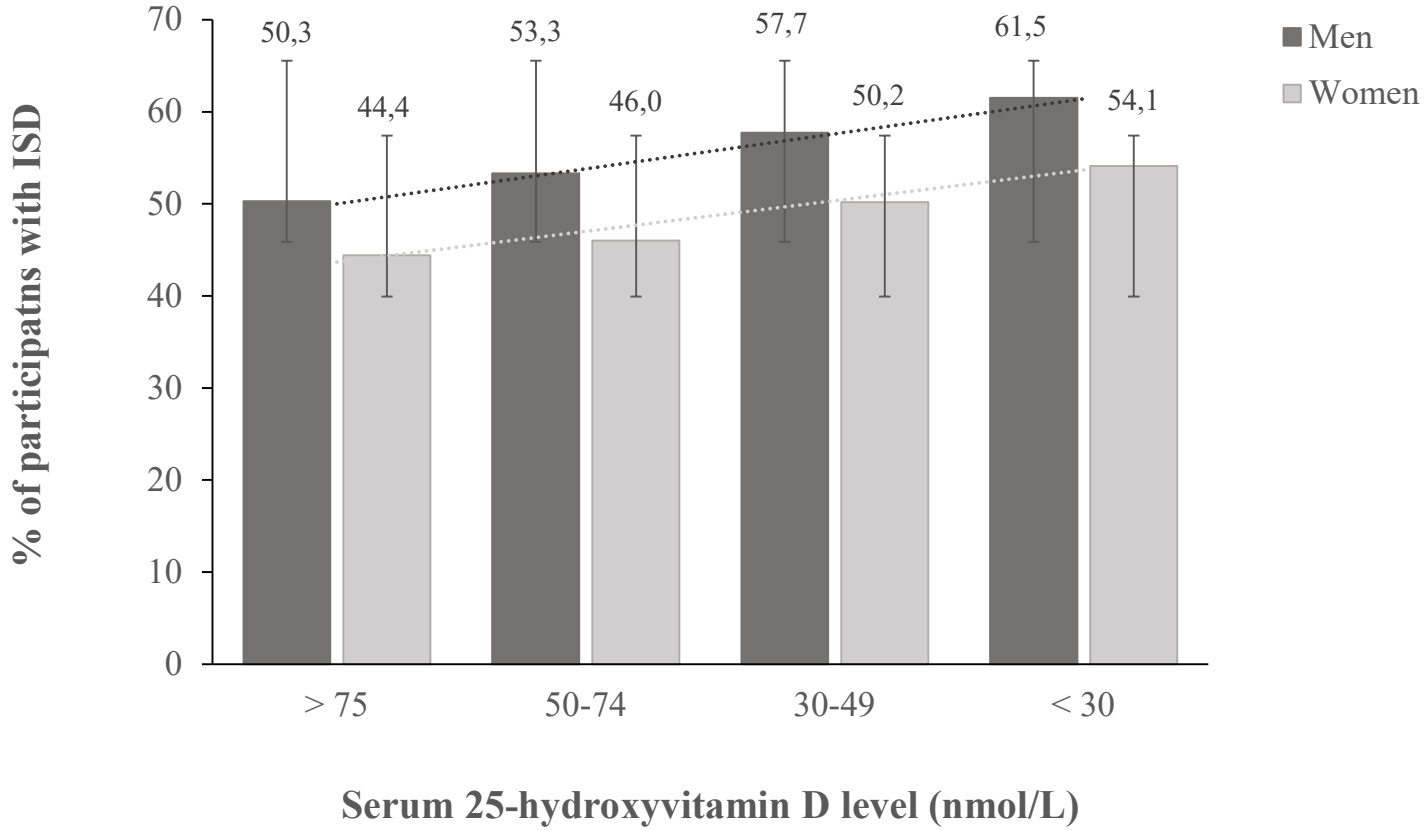


Figure 2. Prevalence of inadequate sleep duration (ISD) across categories of serum 25-hydroxyvitamin D level in men and women. The Tromsø Study 2015-2016.

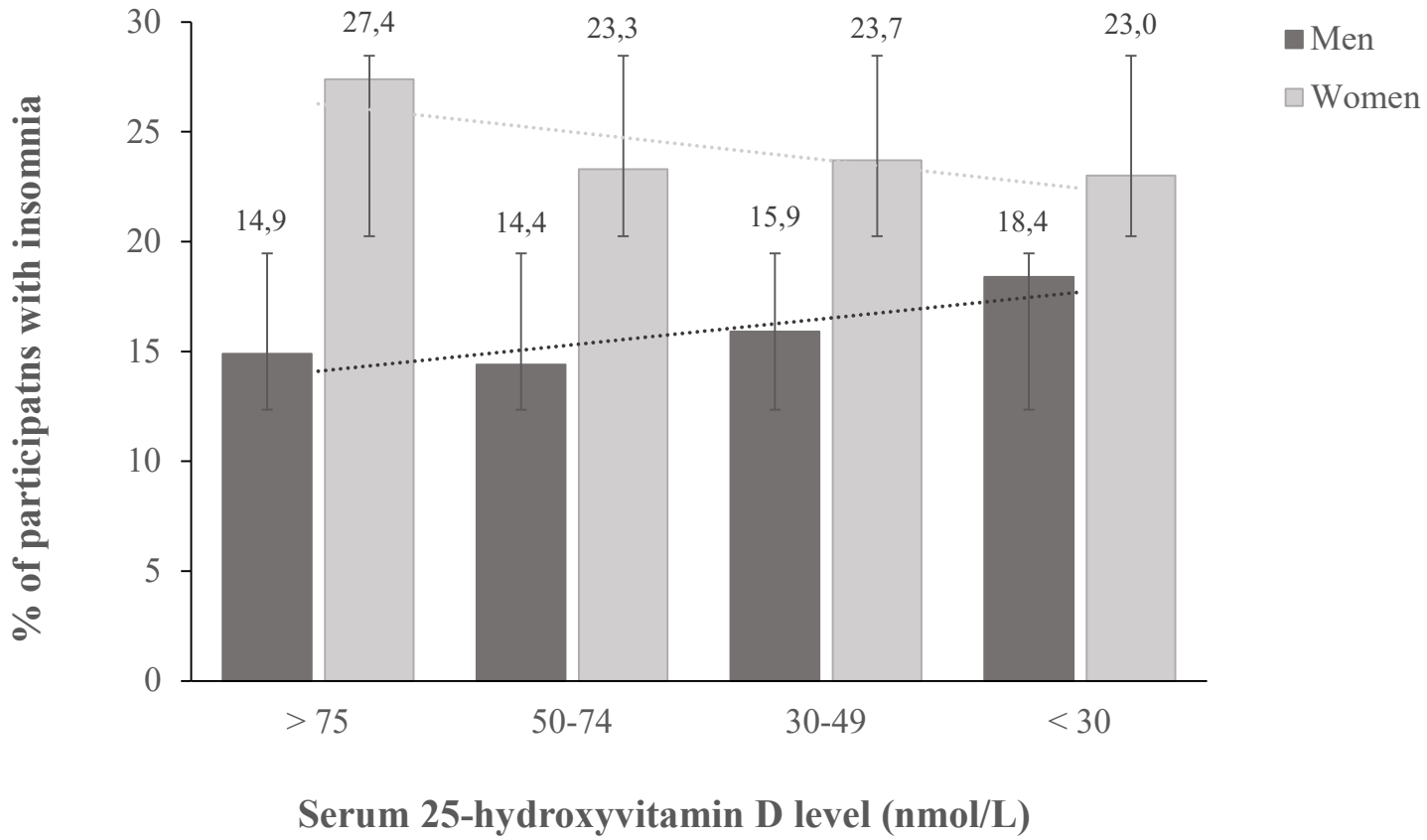


Figure 3. Prevalence of insomnia across categories of serum 25-hydroxyvitamin D level in men and women. The Tromsø Study 2015-2016.

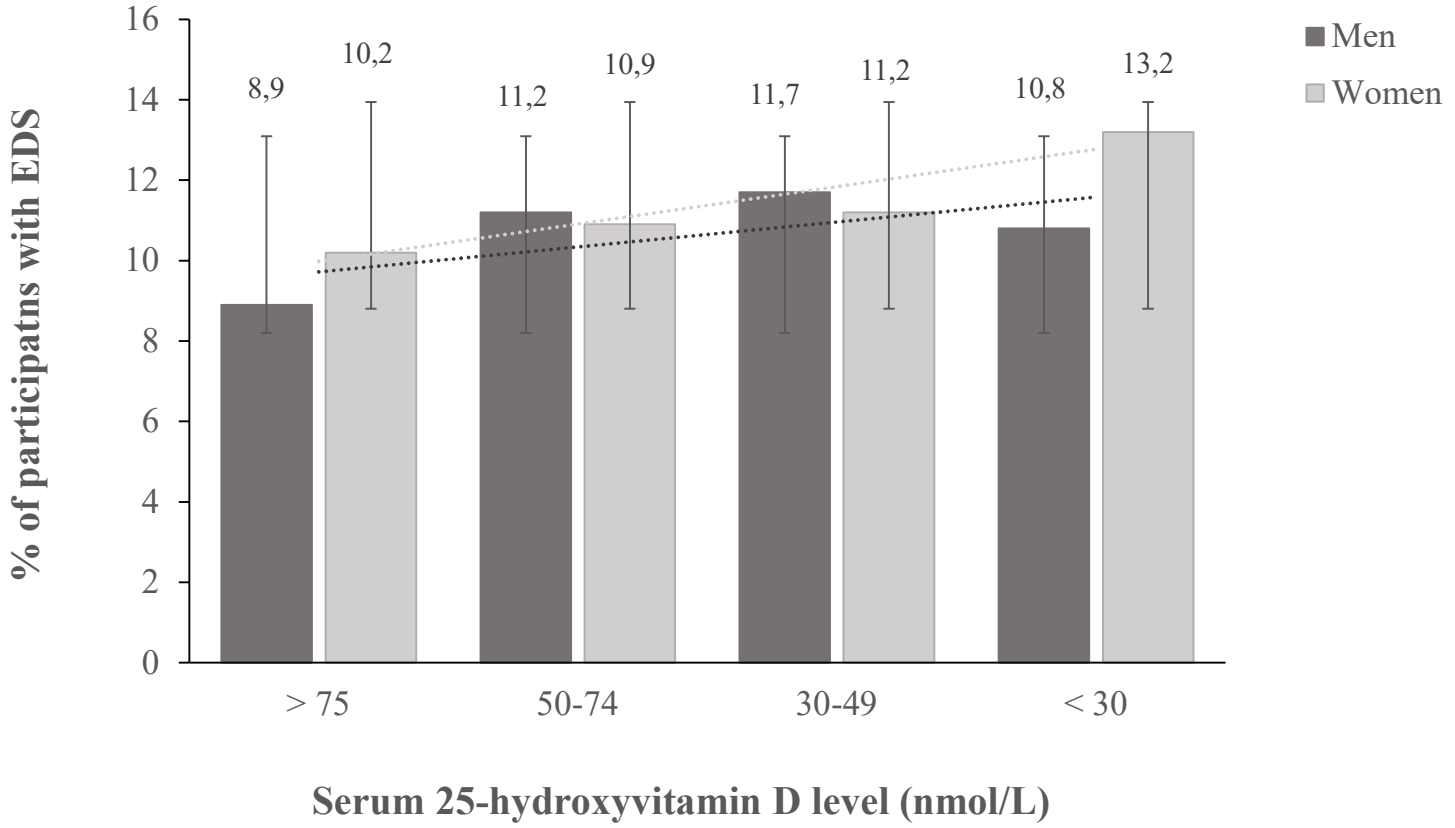


Figure 4. Prevalence of excessive daytime sleepiness (EDS) across categories of serum 25-hydroxyvitamin D level in men and women. The Tromsø Study 2015-2016.

Table 1. Participant characteristics. The Tromsø Study 2015-2016.

	Overall	Excluded		Serum 25-hydroxyvitamin D (nmol/L)				*p*-value
	(N=20,438)	(n=645)		Deficient	Insufficient	Sufficient	High	for difference
				< 30 (2.2-29.9)	30.0-49.9	50.0-75.0	> 75 (75.1-243.2)	
				(n=920)	(n=4,854)	(n=8,996)	(n=5,668)	
Sex, N (%) women	10,713 (52.4)	361 (56.0)		415 (45.1)	2,191 (45.1)	4,671 (51.9)	3,436 (60.6)	.001[a]
Age, years (mean (SD))	57.1 (11.3)	63.6 (13.9)	*	51.1 (9.4)	53.6 (10.3)	57.3 (11.3)	60.7 (11.0)	.001[b]
BMI, kg/m^2 (mean (SD))	27.3 (4.5)	27.7 (4.9)		28.9 (5.8)	28.1 (4.8)	27.3 (4.4)	26.4 (4.1)	.001[b]
BMI-category								.001[a]
<18.5 kg/m^2	111 (0.5)	8 (1.3)		5 (0.5)	22 (0.5)	36 (0.4)	48 (0.8)	
18.5-25 kg/m^2	6,543 (32.1)	185 (29.0)		236 (25.7)	1,272 (26.3)	2,825 (31.5)	2,210 (39.1)	
25-30 kg/m^2	8,899 (43.7)	273 (42.8)		344 (37.5)	2,104 (43.5)	4,046 (45.1)	2,405 (42.5)	
≥30 kg/m^2	4,829 (23.7)	172 (27.0)		332 (36.2)	1,438 (29.7)	2,068 (23.0)	991 (17.5)	
s-25(OH)D, nmol/L (mean (SD))	63.6 (22.0)	62.2 (24.1)		24.5 (4.5)	41.4 (5.5)	62.3 (7.1)	90.8 (14.6)	.001[b]
Season								.001[a]
Winter (October-March)	8,346 (40.8)	210 (43.4)		479 (52.1)	2,165 (44.6)	3,573 (39.7)	2,129 (37.6)	
Summer (April-September)	12,092 (59.2)	274 (56.6)		441 (47.9)	2,689 (55.4)	5,423 (60.3)	3,539 (62.4)	
Living with spouse (yes)	14,893 (77.1)	390 (67.9)	*	567 (66.1)	3,560 (77.2)	6,680 (78.6)	4,086 (76.3)	.001[a]
Self-perceived economy								.001[a]
Average or good	19,652 (96.5)	328 (95.3)		842 (91.7)	4,626 (95.8)	8,707 (97.0)	5,477 (97.0)	
Difficult	721 (3.5)	16 (4.7)		76 (8.3)	202 (4.2)	273 (3.0)	170 (3.0)	
Current smoker (yes)	2,804 (13.8)	100 (15.9)		199 (21.8)	810 (16.8)	1,122 (12.6)	673 (12.0)	.001[a]
Alcohol intake, g/day (mean (SD))	11.4 (13.2)	7.9 (13.1)	*	9.9 (14.2)	10.6 (12.8)	11.3 (13.2)	12.3 (13.4)	.001[b]
Leisure time physical activity								.001[a]
Sedentary or low	14,962 (75.6)	457 (79.8)	*	736 (82.3)	3,662 (77.7)	6,558 (75.2)	4,006 (73.3)	
Moderate or vigorous	4,835 (24.4)	116 (20.2)	*	158 (17.7)	1,051 (22.3)	2,168 (24.8)	1,458 (26.7)	
Shift work (yes)	2,061 (10.4)	17 (6.4)	*	107 (11.8)	530 (11.2)	936 (10.7)	488 (8.9)	.001[a]
OSA (yes)	751 (3.7)	11 (3.6)		51 (5.6)	233 (4.8)	285 (3.2)	182 (3.2)	.001[a]
CVD (yes)	2,586 (13.2)	138 (23.8)	*	100 (11.3)	544 (11.7)	1,137 (13.1)	805 (14.9)	.001[a]
Current cancer (yes)	375 (1.9)	29 (4.8)	*	12 (1.3)	59 (1.2)	173 (2.0)	131 (2.4)	.001[a]
Chronic pain ≥3 months or more	6,957 (37.5)	217 (42.0)	*	352 (41.0)	1,648 (37.0)	3,016 (36.9)	1,941 (38.6)	.033[a]
HSCL-10 ≥1.85	1,705 (8.7)	17 (7.0)		108 (12.3)	448 (9.6)	712 (8.2)	437 (8.1)	.001[a]
Night sweats (sometimes/definitely)	5,040 (48.1)	75 (42.4)		174 (43.1)	932 (43.3)	2,177 (47.5)	1,757 (52.5)	.001[a, c]

Abbreviations: SD = standard deviation; BMI = body mass index; s-25(OH)D = serum 25-hydroxyvitamin D; OSA = obstructive sleep apnoea; CVD = cardiovascular disease; HSCL-10 = Hopkins Symptoms Check List 10.

Values are number of participants (% of participants in that category) if not otherwise specified.

Each characteristic was compared within each vitamin D group using:

[a] Pearson's Chi-square test for categorical variables.

[b] One-way analysis of variance for continuous variables.

[c] Including women only.

Characteristics of excluded participants that were significantly different from the overall sample are denoted *

Table 2. Characteristics of sleep outcomes in men and women. The Tromsø Study (2015-2016).

	Missing data	Overall	Serum 25-hydroxyvitamin D (nmol/L)				*p*-value	
		(N=20,438)	Deficient	Insufficient	Sufficient	High	for difference	
			< 30.0 (2.2-29.9)	30.0-49.9	50.0-75.0	> 75.0 (75.1-243.2)		
Men (n=9,725)			(n=505)	(n=2,663)	(n=4,325)	(n=2,232)		
Sleep duration, minutes (mean (SD))	(n=503)	415.8 (64.6)	408.4 (76.3)	410.3 (65.6)	417.5 (62.9)	420.8 (63.1)	<.001[b]	*
ISD (yes)		4,972 (53.9)	300 (61.1)	1,454 (57.5)	2,174 (53.0)	1,044 (49.7)	<.001[a]	*
Sleep duration category							<.001[a]	*
<5 hours		324 (3.5)	24 (4.9)	104 (4.1)	132 (3.2)	64 (3.0)		
5-7 hours		4,497 (48.8)	263 (53.6)	1,312 (51.9)	1,975 (48.2)	947 (45.1)		
7-9 hours		4,250 (46.1)	191 (38.9)	1,075 (42.5)	1,927 (47.0)	1,057 (50.3)		
>9 hours		151 (1.6)	13 (2.6)	38 (1.5)	67 (1.6)	33 (1.6)		
Insomnia (yes)	(n=340)	1,423 (15.2)	89 (18.4)	408 (15.9)	604 (14.4)	322 (14.9)	.071[a]	
ESS-score	(n=498)	5.9 (3.5)	5.9 (3.5)	6.0 (3.6)	5.9 (3.6)	5.8 (3.4)	.139[a]	
EDS (yes)	(n=498)	995 (10.8)	52 (10.8)	298 (11.7)	458 (11.2)	187 (8.9)	.015[a]	*
Women (n=10,713)			(n=415)	(n=2,191)	(n=4,671)	(n=3,436)		
Sleep duration, minutes (mean (SD))	(n=738)	427.4 (66.2)	417.3 (68.3)	422.4 (61.3)	428.1 (64.9)	430.8 (70.4)	<.001[b]	*
ISD (yes)		4,568 (45.8)	207 (52.8)	1,021 (49.5)	1,984 (45.3)	1,356 (43.2)	<.001[a]	*
Sleep duration category							<.001[a]	*
<5 hours		280 (2.8)	15 (3.8)	55 (2.7)	117 (2.7)	93 (3.0)		
5-7 hours		3,977 (39.9)	178 (45.4)	912 (44.3)	1,731 (39.5)	1,156 (36.8)		
7-9 hours		5,407 (54.2)	185 (47.2)	1,040 (50.5)	2,397 (54.7)	1,785 (56.8)		
>9 hours		311 (3.1)	14 (3.6)	54 (2.6)	136 (3.1)	107 (3.4)		
Insomnia (yes)	(n=561)	2,507 (24.7)	88 (23.0)	493 (23.7)	1,038 (23.3)	888 (27.4)	<.001[a]	*
ESS-score	(n=630)	5.8 (3.7)	6.0 (3.7)	6.0 (3.7)	5.8 (3.7)	5.6 (3.7)	<.001[a]	
EDS (yes)	(n=630)	1,090 (10.8)	52 (13.2)	231 (11.2)	480 (10.9)	327 (10.2)	.258[a]	

Abbreviations: SD = standard deviation; ISD = inadequate sleep duration; ESS = epworth sleepiness scale; EDS = excessive daytime sleepiness.

Sleep duration was calculated as time in bed (calculated as bedtime minus rise time) minus sleep onset latency. ISD was defined as sleeping <7 hours or ≥9 hours. ESS-scores were calculated as the sum of 8 items (scored from 0-3) assessing the probability of napping in everyday situations. EDS was defined as an ESS-score of >10 points. Insomnia was defined as a) having difficulties with initiating sleep, maintaining sleep and/or early morning awakening ≥3 nights per week, b) experiencing daytime sleepiness/tiredness and/or a predominant complaint of dissatisfaction with sleep quantity or quality ≥3 days per week, and c) symptoms being present for ≥3 months.

Values represent numbers and percentages within categories, if not otherwise specified. Each characteristic was compared across the serum 25-hydroxyvitamin D groups using:

[a] Pearson's Chi-square test for categorical variables.

[b] One-way analysis of variance for continuous variables.
Significant associations are denoted*

Table 3. The odds ratio for having ISD, insomnia or EDS in men and women in relation to serum 25-hydroxyvitamin D.

	ISD					Insomnia					EDS				
	s-25(OH)D (continuous)					s-25(OH)D (continuous)					s-25(OH)D (continuous)				
	n	OR	(95% CI)	*p*-value		n	OR	(95% CI)	*p*-value		n	OR	(95% CI)	*p*-value	
Men (n=9,725)															
Model 1[a]	9,222	0.993	0.991, 0.995	<.001	*	9,385	0.998	0.995, 1.001	.132		9,227	0.996	0.993, 0.999	.014	*
Model 2[b]	7,364	0.997	0.995, 0.999	.015	*	7,410	1.003	1.000, 1.007	.068		7,348	0.999	0.995, 1.003	.522	
Women (n=10,713)															
Model 1[a]	9,975	0.996	0.994, 0.997	<.001	*	10,152	1.005	1.003, 1.007	<.001	*	10,083	0.996	0.993, 0.999	.011	*
Model 2[b, c]	7,376	0.998	0.996, 1.000	.067		7,378	1.004	1.001, 1.007	.009	*	7,383	1.001	0.998, 1.005	.471	

Abbreviations: s-25(OH)D = serum 25-hydroxyvitamin D; OR = odds ratio; CI = confidence interval; ISD = inadequate sleep duration; EDS = excessive daytime sleepiness; HSCL-10 = Hopkins Symptoms Check List 10.

Self-reported sleep duration was calculated as time in bed (calculated as bedtime minus rise time) minus sleep onset latency. ESS (Epworth Sleepiness Scale)-scores were calculated as the sum of 8 items (scored from 0-3) assessing the probability of napping in everyday situations. EDS was defined as an ESS-score of >10 points.

OR (95% CI) was calculated for each categorical sleep measure using binary logistic regression with s-25(OH)D as a continuous predictor variable:

[a] Adjusted for month of serum sampling.

[b] Adjusted for month of serum sampling, age, BMI, smoking, alcohol intake, leisure time physical activity, living with a spouse or partner, self-perceived economy, shift work, obstructive sleep apnoea, psychological distress (HSCL-10 ≥1.85) or presence of other comorbidities (cardiovascular disease, current cancer and/or chronic pain during the last 3 months or more).

[c] Adjusted for afflictions of night sweats in women, in addition to other variables.

Significant associations are denoted*

Table 4. The odds ratio for having ISD, insomnia or EDS in men and women by serum 25-hydroxyvitamin D levels.

	Serum 25-hydroxyvitamin D (nmol/L)												
	Deficiency			Insufficiency				Sufficient		High			
	< 30.0 (2.2-29.9)			30.0-49.9				50.0-75.0		> 75.0 (75.1-243.2)			
	n	OR (95% CI)	*p*	n	OR (95% CI)	*p*		n	OR (95% CI)	n	OR (95% CI)	*p*	
Men (n=9,725)													
ISD													
Model 1[a]	491	1.45 (1.19, 1.76)	<.001	*	2,529	1.22 (1.10, 1.35)	<.001	*	4,101	1.00 (Reference)	2,101	0.88 (0.80, 0.98)	.021	*
Model 2[b]	403	1.16 (0.93, 1.44)	.200		2,043	1.10 (0.98, 1.23)	.122		3,279	1.00 (Reference)	1,639	1.01 (0.89, 1.14)	.889	
Insomnia													
Model 1[a]	484	1.28 (0.99, 1.64)	.056		2,558	1.11 (0.97, 1.27)	.145		4,189	1.00 (Reference)	2,154	1.06 (0.91, 1.22)	.466	
Model 2[b]	393	0.99 (0.72, 1.36)	.953		2,045	1.00 (0.84, 1.18)	.951		3,307	1.00 (Reference)	1,665	1.19 (0.99, 1.43)	.062	
EDS													
Model 1[a]	480	1.00 (0.73, 1.36)	.982		2,544	1.07 (0.91, 1.25)	.409		4,105	1.00 (Reference)	2,098	0.78 (0.65, 0.93)	.006	*
Model 2[b]	394	0.93 (0.65, 1.32)	.681		2,057	1.04 (0.87, 1.25)	.647		3,262	1.00 (Reference)	1,635	0.86 (0.70, 1.06)	.148	
Women (n=10,713)													
ISD													
Model 1[a]	392	1.40 (1.14, 1.73)	.001	*	2,061	1.18 (1.07, 1.32)	.002	*	4,381	1.00 (Reference)	3,141	0.93 (0.85, 1.02)	.124	
Model 2[b, c]	288	1.23 (0.95, 1.57)	.111		1,574	1.16 (1.03, 1.32)	.017	*	3,285	1.00 (Reference)	2,229	1.02 (0.91, 1.14)	.766	
Insomnia													
Model 1[a]	382	0.97 (0.76, 1.24)	.804		2,082	1.01 (0.89, 1.14)	.869		4,448	1.00 (Reference)	3,240	1.25 (1.13, 1.38)	<.001	*
Model 2[b, c]	276	0.84 (0.60, 1.17)	.298		1,557	1.07 (0.92, 1.26)	.388		3,289	1.00 (Reference)	2,256	1.16 (1.01, 1.33)	.036	*
EDS													
Model 1[a]	394	1.27 (0.93, 1.73)	.126		2,063	1.05 (0.89, 1.24)	.597		4,411	1.00 (Reference)	3,215	0.93 (0.80, 1.07)	.306	
Model 2[b, c]	287	1.01 (0.70, 1.45)	.962		1,563	0.91 (0.75, 1.11)	.354		3,274	1.00 (Reference)	2,259	1.05 (0.88, 1.25)	.623	

Abbreviations: OR = odds ratio; CI = confidence interval; ISD = inadequate sleep duration; EDS = excessive daytime sleepiness; HSCL-10 = Hopkins Symptoms Check List 10.

Sleep duration was calculated as time in bed (calculated as self-reported bedtime minus self-reported rise time) minus self-reported sleep onset latency. ISD was defined as sleeping <7 hours or ≥9 hours. ESS-scores were calculated as the sum of 8 items (scored from 0-3) assessing the probability of napping in everyday situations. EDS was defined as an ESS-score (Epworth Sleepiness Scale) of >10 points. Insomnia was defined as a) having difficulties with initiating sleep, maintaining sleep and/or early morning awakening ≥3 nights per week, b) experiencing daytime sleepiness/tiredness and/or a predominant complaint of dissatisfaction with sleep quantity or quality ≥3 days per week, and c) symptoms being present for ≥3 months.

OR (95% CI) was calculated for each categorical sleep measure using binary logistic regression with s-25(OH)D level as a categorical predictor variable:

[a] Adjusted for month of serum sampling.

[b] Adjusted for month of serum sampling, age, BMI, smoking, alcohol intake, leisure time physical activity, living with a spouse or partner, self-perceived economy, shift work, obstructive sleep apnoea, psychological distress (HSCL-10 ≥1.85) or presence of other comorbidities (cardiovascular disease, current cancer and/or chronic pain during the last 3 months or more).
[c] Adjusted for afflictions of night sweats in women, in addition to other variables.
Significant associations are denoted*

Table 5. Associations of serum 25 hydroxyvitamin D level (continuous) with sleep duration and ESS-score.

	Sleep duration (minutes)					ESS-score				
	n	Unstandardized β	(95% CI)	*p*-value		n	Unstandardized β	(95% CI)	*p*-value	
Men (n=9,725)										
Model 1[a]	9,222					9,227				
Deficient (< 30 nmol/L)		-10.05	(-16.15, -3.95)	.001	*		-0.085	(-0.423, 0.253)	.622	
Insufficient (30-49 nmol/L)		-7.23	(-10.95, -4.51)	<.001	*		0.071	(-0.105, 0.247)	.431	
Sufficient (50-75 nmol/L)		1.00	(Reference)	.			1.000	(Reference)	.	
High (> 75 nmol/L)		3.50	(0.10, 6.90)	.043	*		-0.159	(-0.345, 0.027)	.095	
Model 2[b]	7,376					7,362				
Deficient (< 30 nmol/L)		-4.83	(-11.37, 1.72)	.148			-0.063	(-0.434, 0.308)	.739	
Insufficient (30-49 nmol/L)		-6.55	(-10.01, -3.08)	<.001	*		0.103	(-0.92, 0.297)	.300	
Sufficient (50-75 nmol/L)		1.00	(Reference)	.			1.000	(Reference)	.	
High (> 75 nmol/L)		2.72	(-0.98, 6.41)	.149			-0.094	(-0.302, 0.113)	.373	
Women (n=10,713)										
Model 1[a]	9,975					10,083				
Deficient (< 30 nmol/L)		-9.14	(-16.01, -2.28)	.009	*		0.202	(-0.179, 0.584)	.298	
Insufficient (30-49 nmol/L)		-5.77	(-9.24, 2.29)	.001	*		0.196	(0.003, 0.389)	.047	*
Sufficient (50-75 nmol/L)		1.00	(Reference)	.			1.000	(Reference)	.	
High (> 75 nmol/L)		2.77	(-0.27, 5.81)	.074			-0.225	(-0.393, -0.057)	.009	*
Model 2[b, c]	7,391					7,398				
Deficient (< 30 nmol/L)		-6.86	(-14.50, 0.78)	0.78			0.227	(-0.217, 0.671)	.317	
Insufficient (30-49 nmol/L)		-6.62	-10.42, -2.83)	<.001	*		0.216	(-0.005, 0.437)	.055	
Sufficient (50-75 nmol/L)		1.00	(Reference)	.			1.000	(Reference)	.	
High (> 75 nmol/L)		2.28	(-1.13, 5.68)	.198			-0.276	(-0.473, -0.079)	.006	*

Abbreviations: OR = odds ratio; CI = confidence interval; ESS = epworth sleepiness scale; s-25(OH)D = serum 25 hydroxyvitamin D.

Sleep duration was calculated as time in bed (calculated as self-reported bedtime minus self-reported rise time) minus self-reported sleep onset latency. ESS-scores were calculated as the sum of 8 items (scored from 0-3) assessing the probability of napping in everyday situations.

Unstandardized β -values (95% CI) are reported separately for each s-25(OH)D group (deficient, insufficient, sufficient, high) and were calculated using multiple linear regression using the s-25(OH)D sufficient group as the reference category:

[a] Adjusted for month of serum sampling.

[b] Adjusted for month of serum sampling, age, BMI, smoking, alcohol intake, leisure time physical activity, living with a spouse or partner, self-perceived economy, shift work, obstructive sleep apnoea, psychological distress (Hopkins Symptoms Check List (HSCL-10) ≥1.85) or presence of other comorbidities (cardiovascular disease, current cancer and/or chronic pain during the last 3 months or more).

[c] Adjusted for afflictions of night sweats in women, in addition to other variables.

Significant associations are denoted*

Supplemental Figure 1. The questionnaire items used to assess habitual sleep traits in the present study, collected in the seventh survey of the Tromsø Study (Tromsø7: 2015-2016).

Sleep duration	When do you usually go to sleep? (bedtime)	00:30-24:00 (dropdown menu)
	For how long are you awake before you fall asleep? (sleep onset latency)	Insert minutes
	What time do you usually wake up? (risetime)	00:30-24:00 (dropdown menu)
Insomnia	How many days a week (Tick the number of days) - Do you usually use more than 30 minutes to fall asleep? - Do you usually wake up for more than 30 minutes in the middle of the night? - Do you wake up 30 minutes earlier than you wished, without being able to go back to sleep again? - Have you not felt refreshed after sleep? - Felt so tired that it has affected your work, school, or private life? - Have you been dissatisfied with your sleep?	Number of days a week 0 1 2 3 4 5 6 7
	If sleep problems, how long time?	< 1 week, 1-3 weeks, 1 month, 2 months, 3 months, 4-6 months, 7-12 months, 1-5 years, 6-10 years, > 10 years, do not have sleep problems
Daytime sleepiness	How likely are you to doze off or fall asleep in the following situation? - Sitting and reading - Watching TV - Sitting inactive in a public place (e.g., a theatre or meeting) - As a passenger in a car for an hour without break - Lying down to rest in the afternoon when circumstances permit - When you are sitting and talking to someone - When you are sitting quietly after lunch without alcohol - When you are in a car, while stopped for a few minutes in traffic	Use the scale from 0-3 for each situation: - 0= No chance of dosing - 1= Slight chance of dozing - 2= Moderate chance of dozing - 3= High chance of dozing
Other	Do you usually work shifts or at night?	- No - Yes
	Have you had breathing pauses (sleep apnea) at sleep?	- Never or less than once per month - Less than once per week - 1-2 nights per week - 3-5 nights per week - Daily or almost daily - Don’t know

Supplemental Table 1. The odds ratio for having ISD, insomnia or EDS in men and women in relation to serum 25-hydroxyvitamin D in **winter**.

	ISD					Insomnia				EDS			
	s-25(OH)D					s-25(OH)D				s-25(OH)D			
	n	OR	(95% CI)	*p*-value		n	OR	(95% CI)	*p*-value	n	OR	(95% CI)	*p*-value
Men (n=4,038)													
Model 1	3,837	0.990	0.987, 0.993	<.001	*	3,886	1.000	0.996, 1.003	.804	3,844	0.996	0.991, 1.001	.109
Model 2[a]	3,044	0.995	0.991, 0.999	.006	*	3,050	1.004	0.999, 1.009	.106	3,038	0.998	0.992, 1.004	.510
Women (n=4,308)													
Model 1	4,024	0.997	0.994, 1.9996	.026	*	4,086	1.006	1.003, 1.009	<.001	4,067	0.999	0.994, 1.003	.581
Model 2[a, b]	2,981	0.998	0.994, 1.002	.282		2,976	1.004	0.999, 1.008	.118	2,986	1.002	0.997, 1.008	.384

Abbreviations: s-25(OH)D = serum 25-hydroxyvitamin D; OR = odds ratio; CI = confidence interval; ISD = inadequate sleep duration; EDS = excessive daytime sleepiness; ESS = Epworth Sleepiness Scale; HSCL-10 = Hopkins Symptoms Check List 10.

Self-reported sleep duration was calculated as time in bed (calculated as bedtime minus rise time) minus sleep onset latency. ISD was defined as sleeping <7 hours or ≥9 hours. Insomnia was defined as a) having difficulties with initiating sleep, maintaining sleep and/or early morning awakening ≥3 nights per week, b) experiencing daytime sleepiness/tiredness and/or a predominant complaint of dissatisfaction with sleep quantity or quality ≥3 days per week, and c) symptoms being present for ≥3 months. EDS was defined as an ESS-score of >10 points.

OR (95% CI) was calculated for each categorical sleep measure using binary logistic regression with s-25(OH)D as a continuous predictor variable:

[a] Adjusted for age, BMI, smoking, alcohol intake, leisure time physical activity, living with a spouse or partner, self-perceived economy, shift work, obstructive sleep apnoea, psychological distress (HSCL-10 ≥1.85) or presence of other comorbidities (cardiovascular disease, current cancer and/or chronic pain during the last 3 months or more).

[b] Adjusted for afflictions of night sweats in women, in addition to other variables.

Significant associations are denoted*

Supplemental Table 2. The odds ratio for having ISD, insomnia or EDS in men and women by serum 25-hydroxyvitamin D levels in **winter**.

	Serum 25-hydroxyvitamin D (nmol/L)												
	Deficiency			Insufficiency				Normal		High			
	< 30.0 (2.2-29.9)			30.0-49.9				50.0-75.0		> 75.0 (75.1-243.2)			
	n	OR (95% CI)	*p*	n	OR (95% CI)	*p*		n	OR (95% CI)	n	OR (95% CI)	*p*	
Men (n=4,038)													
ISD													
Model 1	281	1.88 (1.44, 2.46)	<.001	*	1,156	1.31 (1.13, 1.53)	<.001	*	1,624	1.00 (Reference)	776	0.88 (0.74, 1.04)	.130	
Model 2[a]	225	1.49 (1.10, 2.02)	.011	*	934	1.21 (1.02, 1.44)	.032	*	1,293	1.00 (Reference)	592	1.03 (0.84, 1.25)	.795	
Insomnia													
Model 1	275	1.11 (0.80, 1.55)	.524		1,172	0.95 (0.78, 1.16)	.632		1,649	1.00 (Reference)	790	1.00 (0.80, 1.25)	.991	
Model 2[a]	218	0.91 (0.59, 1.39)	.656		935	0.85 (0.65, 1.09)	.196		1,300	1.00 (Reference)	597	1.12 (0.84, 1.49)	.429	
EDS													
Model 1	277	1.04 (0.69, 1.56)	.859		1,169	1.04 (0.82, 1.32)	.754		1,625	1.00 (Reference)	773	0.77 (0.57, 1.03)	.082	
Model 2[a]	225	1.09 (0.68, 1.75)	.720		939	1.05 (0.79, 1.39)	.729		1,284	1.00 (Reference)	590	0.91 (0.65, 1.27)	.564	
Women (n=4,308)													
ISD													
Model 1	186	1.41 (1.04, 1.91)	.026	*	889	1.26 (1.07, 1.48)	.005	*	1,754	1.00 (Reference)	1,195	1.04 (0.90, 1.21)	.605	
Model 2[a, b]	139	1.38 (0.96, 1.98)	.083		693	1.32 (1.09, 1.60)	.005	*	1,317	1.00 (Reference)	832	1.15 (0.96, 1.38)	.134	
Insomnia													
Model 1	179	0.81 (0.56, 1.18)	.278		898	0.99 (0.82, 1.19)	.883		1,777	1.00 (Reference)	1,232	1.28 (1.08, 1.50)	.004	*
Model 2[a, b]	134	0.79 (0.47, 1.33)	.374		681	1.16 (0.91, 1.48)	.236		1,323	1.00 (Reference)	838	1.13 (0.91, 1.41)	.278	
EDS													
Model 1	185	1.18 (0.74, 1.89)	.488		893	1.08 (0.83, 1.40)	.568		1,765	1.00 (Reference)	1,224	0.99 (0.78, 1.26)	.912	
Model 2[a, b]	139	0.85 (0.49, 1.50)	.583		683	1.00 (0.74, 1.34)	.981		1,318	1.00 (Reference)	846	1.06 (0,79, 1.41)	.715	

Abbreviations: OR = odds ratio; CI = confidence interval; ISD = inadequate sleep duration; EDS = excessive daytime sleepiness; ESS = Epworth Sleepiness Scale; HSCL-10 = Hopkins Symptoms Check List 10. Self-reported sleep duration was calculated as time in bed (calculated as bedtime minus rise time) minus sleep onset latency. ISD was defined as sleeping <7 hours or ≥9 hours. Insomnia was defined as a) having difficulties with initiating sleep, maintaining sleep and/or early morning awakening ≥3 nights per week, b) experiencing daytime sleepiness/tiredness and/or a predominant complaint of dissatisfaction with sleep quantity or quality ≥3 days per week, and c) symptoms being present for ≥3 months. EDS was defined as an ESS-score of >10 points.

OR (95% CI) was calculated for each categorical sleep measure using binary logistic regression with s-25(OH)D level as a categorical predictor variable:

[a] Adjusted for age, BMI, smoking, alcohol intake, leisure time physical activity, living with a spouse or partner, self-perceived economy, shift work, obstructive sleep apnoea, psychological distress (HSCL-10 ≥1.85) or presence of other comorbidities (cardiovascular disease, current cancer and/or chronic pain during the last 3 months or more).

[b] Adjusted for afflictions of night sweats in women, in addition to other variables.
Significant associations are denoted*

Supplemental Table 3. Associations of serum 25-hydroxyvitamin D level (continuous) with sleep duration (continuous) and ESS-score (continuous) in **winter**.

	Sleep duration (minutes)				ESS-score			
	Unstandardized β	95% CI	*p*-value		Unstandardized β	95% CI	*p*-value	
Men (n=4,038)								
Model 1[a]	0.29	0.19, 0.39	<.001	*	0.00	-0.01, 0.00	.137	
Model 2[b]	0.11	0.00, 0.21	.051		0.00	-0.01, 0.01	.987	
Women (n=4,308)								
Model 1[a]	0.20	0.10, 0.29	<.001	*	-0.01	-0.01, -0.004	<.001	*
Model 2[b, c]	0.12	0.02, 0.22	.016	*	0.00	-0.01, 0.01	.982	

Abbreviations: ESS = Epworth Sleepiness Scale; HSCL-10 = Hopkins Symptoms Check List 10.

Self-reported sleep duration was calculated as time in bed (calculated as bedtime minus rise time) minus sleep onset latency. ESS-scores were calculated as the sum of 8 items (scored from 0-3) assessing the probability of napping in everyday situations.

Unstandardized β -values (95% CI) are reported for every 10 nmol/L increase in s-25(OH)D and were calculated using multiple linear regression:

[a] Adjusted for age, BMI, smoking, alcohol intake, leisure time physical activity, living with a spouse or partner, self-perceived economy, shift work, obstructive sleep apnoea, psychological distress (HSCL-10 ≥1.85) or presence of other comorbidities (cardiovascular disease, current cancer and/or chronic pain during the last 3 months or more).

[b] Adjusted for afflictions of night sweats in women, in addition to other variables.

Significant associations are denoted*

Supplemental Table 4. The odds ratio for having ISD, insomnia or EDS in men and women in relation to serum 25-hydroxyvitamin D in **summer**.

	ISD					Insomnia					EDS				
	s-25(OH)D					s-25(OH)D					s-25(OH)D				
	n	OR	(95% CI)	*p*-value		n	OR	(95% CI)	*p*-value		n	OR	(95% CI)	*p*-value	
Men (n=5,687)															
Model 1	5,385	0.995	0.992, 0.997	<.001	*	5,499	0.996	0.992, 1.000	.037	*	5,383	0.996	0.992, 1.000	.072	
Model 2[a]	4,320	0.999	0.996, 1.002	.484		4,360	1.002	0.997, 1.006	.506		4,310	0.999	0.994, 1.004	.765	
Women (n=6,405)															
Model 1	5,951	0.995	0.993, 0.997	<.001	*	6,066	1.004	1.001, 1.007	.003	*	6,016	0.995	0.991, 0.999	.008	*
Model 2[a, b]	4,395	0.998	0.995, 1.001	.132		4,402	1.004	1.000, 1.007	.054		4,397	1.000	0.995, 1.004	.979	

Abbreviations: s-25(OH)D = serum 25-hydroxyvitamin D; OR = odds ratio; CI = confidence interval; ISD = inadequate sleep duration; EDS = excessive daytime sleepiness; ESS = Epworth Sleepiness Scale; HSCL-10 = Hopkins Symptoms Check List 10.

Self-reported sleep duration was calculated as time in bed (calculated as bedtime minus rise time) minus sleep onset latency. ISD was defined as sleeping <7 hours or ≥9 hours. Insomnia was defined as a) having difficulties with initiating sleep, maintaining sleep and/or early morning awakening ≥3 nights per week, b) experiencing daytime sleepiness/tiredness and/or a predominant complaint of dissatisfaction with sleep quantity or quality ≥3 days per week, and c) symptoms being present for ≥3 months. EDS was defined as an ESS-score of >10 points.

OR (95% CI) was calculated for each categorical sleep measure using binary logistic regression with s-25(OH)D as a continuous predictor variable:

[a] Adjusted for age, BMI, smoking, alcohol intake, leisure time physical activity, living with a spouse or partner, self-perceived economy, shift work, obstructive sleep apnoea, psychological distress (HSCL-10 ≥1.85) or presence of other comorbidities (cardiovascular disease, current cancer and/or chronic pain during the last 3 months or more).

[b] Adjusted for afflictions of night sweats in women, in addition to other variables.

Significant associations are denoted*

Supplemental Table 5. The odds ratios for having ISD, insomnia or EDS in men and women by serum 25-hydroxyvitamin D levels in **summer**.

	Serum 25-hydroxyvitamin D (nmol/L)											
	Deficiency			Insufficiency			Normal		High			
	< 30.0 (2.2-29.9)			30.0-49.9			50.0-75.0		> 75.0 (75.1-243.2)			
	n	OR (95% CI)	*p*	n	OR (95% CI)	*p*	n	OR (95% CI)	n	OR (95% CI)	*p*	
Men (n=5,687)												
ISD												
Model 1	210	1,02 (0.77, 1.35)	.890	1,373	1,13 (0.99, 1.29)	.071	2,477	1.00 (Reference)	1,325	0.89 (0.78, 1.02)	.088	
Model 2[a]	178	0.85 (0.62, 1.17)	.326	1,109	1.01 (0.87, 1.17)	.908	1,986	1.00 (Reference)	1,047	1.00 (0.86, 1.17)	.966	
Insomnia												
Model 1	209	1.52 (1.05, 2.20)	.027 *	1,386	1.27 (1.05, 1.53)	.012 *	2,540	1.00 (Reference)	1,364	1.09 (0.90, 1.33)	.365	
Model 2[a]	175	1.16 (0.73, 1.84)	.542	1,110	1.16 (0.92, 1.46)	.224	2,007	1.00 (Reference)	1,068	1.23 (0.97, 1.56)	.085	
EDS												
Model 1	203	0.90 (0.56, 1.44)	.657	1,375	1.08 (0.88, 1.32)	.473	2,480	1.00 (Reference)	1,325	0.78 (0.63, 0.98)	.033 *	
Model 2[a]	169	0.79 (0.46, 1.35)	.386	1,118	1.05 (0.83, 1.32)	.710	1,978	1.00 (Reference)	1,045	0.85 (0.66, 1.10)	.210	
Women (n=6,405)												
ISD												
Model 1	206	1.37 (1.03, 1.83)	.029 *	1,172	1.13 (0.99, 1.30)	.081	2,627	1.00 (Reference)	1,946	0.88 (0.78, 0.98)	.026 *	
Model 2[a, b]	149	1.14 (0.81, 1.60)	.468	881	1.08 (0.92, 1.27)	.344	1,968	1.00 (Reference)	1,397	0.95 (0.83, 1.10)	.516	
Insomnia												
Model 1	203	1.14 (0.82, 1.59)	.426	1,184	1.04 (0.89, 1.22)	.630	2,671	1.00 (Reference)	2,008	1.22 (1.07, 1.40)	.003 *	
Model 2[a, b]	142	0.90 (0.58, 1.40)	.635	876	1.03 (0.84, 1.27)	.775	1,966	1.00 (Reference)	1,418	1.17 (0.98, 1.39)	.087	
EDS												
Model 1	209	1.32 (0.88, 1.97)	.184	1,170	1.01 (0.81, 1.25)	.952	2,646	1.00 (Reference)	1,991	0.89 (0.74, 1.08)	.231	
Model 2[a, b]	148	1.19 (0.74, 1.91)	.480	880	0.87 (0.67, 1.12)	.275	1,956	1.00 (Reference)	1,413	1.02 (0.82, 1.28)	.842	

Abbreviations: OR = odds ratio; CI = confidence interval; ISD = inadequate sleep duration; EDS = excessive daytime sleepiness; ESS = Epworth Sleepiness Scale; HSCL-10 = Hopkins Symptoms Check List 10. Self-reported sleep duration was calculated as time in bed (calculated as bedtime minus rise time) minus sleep onset latency. ISD was defined as sleeping <7 hours or ≥9 hours. Insomnia was defined as a) having difficulties with initiating sleep, maintaining sleep and/or early morning awakening ≥3 nights per week, b) experiencing daytime sleepiness/tiredness and/or a predominant complaint of dissatisfaction with sleep quantity or quality ≥3 days per week, and c) symptoms being present for ≥3 months. EDS was defined as an ESS-score of >10 points.

OR (95% CI) was calculated for each categorical sleep measure using binary logistic regression with s-25(OH)D level as a categorical predictor variable:

[a] Adjusted for age, BMI, smoking, alcohol intake, leisure time physical activity, living with a spouse or partner, self-perceived economy, shift work, obstructive sleep apnoea, psychological distress (HSCL-10 ≥1.85) or presence of other comorbidities (cardiovascular disease, current cancer and/or chronic pain during the last 3 months or more).

[b] Adjusted for afflictions of night sweats in women, in addition to other variables.
Significant associations are denoted*

Supplemental Table 6. Associations of serum 25-hydroxyvitamin D level (continuous) with sleep duration (continuous) and ESS-score (continuous) in **summer**.

	Sleep duration (minutes)				ESS-score			
	Unstandardized β	95% CI	*p*-value		Unstandardized β	95% CI	*p*-value	
Men (n=5,687)								
Model 1[a]	0.17	0.09, 0.25	<.001	*	0.00	-0.01, 0.00	.411	
Model 2[b]	0.02	-0.07, 0.11	.658		0.00	0.00, 0.01	.273	
Women (n=6,405)								
Model 1[a]	0.15	0.08, 0.23	<.001	*	-0.01	-0.01, -0.004	<.001	*
Model 2[b, c]	0.06	-0.02, 0.14	.161		0.01	0.00, 0.01	.792	

Abbreviations: ESS = Epworth Sleepiness Scale; HSCL-10 = Hopkins Symptoms Check List 10.

Self-reported sleep duration was calculated as time in bed (calculated as bedtime minus rise time) minus sleep onset latency. ESS-scores were calculated as the sum of 8 items (scored from 0-3) assessing the probability of napping in everyday situations.

Unstandardized β -values (95% CI) are reported for every 10 nmol/L increase in s-25(OH)D and were calculated using multiple linear regression:

[a] Adjusted for age, BMI, smoking, alcohol intake, leisure time physical activity, living with a spouse or partner, self-perceived economy, shift work, obstructive sleep apnoea, psychological distress (HSCL-10 ≥1.85) or presence of other comorbidities (cardiovascular disease, current cancer and/or chronic pain during the last 3 months or more).

[b] Adjusted for afflictions of night sweats in women, in addition to other variables.

Significant associations are denoted*

Paper II

Larsen AU, Hopstock LA, Jorde R, Grimnes G.

No improvement of sleep from vitamin D supplementation: insights from a randomized controlled trial.

Sleep medicine: X 2021. 3:100040.

Sleep Medicine: X 3 (2021) 100040

Contents lists available at ScienceDirect

Sleep Medicine: X

journal homepage: www.elsevier.com/locate/sleep

No improvement of sleep from vitamin D supplementation: insights from a randomized controlled trial

A.U. Larsen [a, *], L.A. Hopstock [b], R. Jorde [a], G. Grimnes [a, c]

[a] Department of Clinical Medicine, UiT The Arctic University of Norway, Tromsø, Norway
[b] Department of Community Medicine, UiT The Arctic University of Norway, Tromsø, Norway
[c] Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway

ARTICLE INFO

Article history:
Received 16 June 2021
Received in revised form
11 October 2021
Accepted 13 October 2021
Available online 19 October 2021

Keywords:
Sleep duration
Excessive daytime sleepiness
Insomnia
Vitamin D
RCT

ABSTRACT

Background: Vitamin D has been linked to sleep health in observational studies. Data from randomized controlled trials (RCTs) with vitamin D is scarce.
Methods: This study presents the results of a secondary analysis of 189 vitamin D insufficient participants (47.1% women) in a previously performed RCT, of which 92 were randomized to vitamin D (100,000 IU (2500 μg) as a bolus dose followed by 20,000 IU (500 μg) per week), and 97 to placebo. At baseline and after 4 months at the end of the study serum 25-hydroxyvitamin D (s-25(OH)D) was measured, and the study questionnaire assessing sleep duration, daytime sleepiness, and symptoms of insomnia, was completed.
Results: At baseline, mean s-25(OH)D was 35.0 ± 11.8 and 35.5 ± 13.3 nmol/L in the vitamin D and placebo groups, respectively. After four months, we found no statistically significant differences between the intervention groups in any of the assessed sleep outcomes, neither when stratified by sex, nor when performed in subgroups based on baseline or end of study s-25(OH)D level or presence of sleep complaints at baseline.
Conclusions: We were not able to demonstrate a significant effect of vitamin D supplementation on sleep in this vitamin D insufficient population.

1. Introduction

Sleep is a universal, recurring, and reversible physiological state with restorative and transformative effects, promoting optimal health and well-being [1]. Growing evidence suggest that sleep habits are important, modifiable risk factors for non-communicable diseases [2] which may significantly impact quality of life and overall health status. Specifically, poor sleep health has been associated with metabolic [3], endocrine [4] and psychiatric [5] diseases, as well as all-cause mortality [6]. Combined with high treatment-related costs from increased health care utilization [7], it is therefore concerning that the prevalence of sleep disorders has become epidemic [8].

A growing literature suggest a role of vitamin D in maintaining optimal sleep [9–11], in addition to its well-established role in the preservation of calcium and phosphorus homeostasis [12]. Expression of key enzymes required for vitamin D metabolism, activation and degradation have been demonstrated in brain cells, suggesting local production and regulation of vitamin D for auto- and/or paracrine purposes [13,14]. In particular, this involves the 25-hydroxylases responsible for the conversion of vitamin D to its main circulating metabolite 25-hydroxyvitamin D (25(OH)D), the 1α-hydroxylase responsible for the activating step forming the vitamin D hormone 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), capable of binding the vitamin D receptor (VDR), and the 24-hydroxylase necessary in the first step of inactivation and degradation of vitamin D. Notably, the expression of these enzymes was found to be strongest in the hypothalamus and substantia nigra, areas known to be involved in sleep regulation in humans [13]. Thus, as a fat-soluble, steroid hormone, capable of crossing the blood brain barrier [15], it is plausible that vitamin D could exert

Abbreviations: RCT, randomized controlled trial; s-25(OH)D, serum 25-hydroxyvitamin D; ISD, inadequate sleep duration; ESS, epworth sleepiness scale; EDS, excessive daytime sleepiness; s-Ca, serum calcium; p-PTH, plasma parathyroid hormone; BMI, body mass index; BDI-II, beck depression inventory II.

* Corresponding author. Department of Clinical Medicine, UiT The Arctic University of Norway, 9037 Tromsø, Norway.
E-mail address: anette.uhlving@post.uit.no (A.U. Larsen).

https://doi.org/10.1016/j.sleepx.2021.100040

direct effects on brain cells in areas of the brain and brainstem involved in the initiation, maintenance, and timing of sleep through binding of the VDR expressed in these areas [13].

Although not yet fully elucidated, several actions of vitamin D potentially involved in the regulatory mechanism of sleep have been proposed. Experimental studies have indicated that vitamin D might be involved in the regulation of central circadian clock genes [13,16,17], as well as in the transduction of light signals that regulate circadian rhythms [18]. Moreover, vitamin D has been suggested to play a pivotal role in the synthesis of the sleep hormone melatonin [10,11] by regulating the expression of the tryptophan hydroxylase (TPH)-2 [19]. TPH-2 converts tryptophan to 5-hydroxytryptophan, which is further metabolized to serotonin, serving as a main substrate for melatonin synthesis [20]. An emerging literature also highlights an interrelation between vitamin D, sleep, and pain [21,22]. Interestingly, in a recent study among U.S. veterans with chronic pain, vitamin D supplementation relieved both pain symptoms and improved sleep [23]. Whether vitamin D represents an indirect role for improved sleep by alleviating pain symptoms or share interrelated pathways in the regulatory mechanisms of both sleep and pain, is currently unknown.

Several observational studies have reported an association between vitamin D deficiency and poor sleep health, including increased risk of short sleep duration [24–26], daytime sleepiness [27,28] and poor sleep quality [29,30]. Nevertheless, a causal link has been difficult to demonstrate, as results from randomized controlled trials (RCTs) are scarce. Previous supplementation trials have shown contrasting results, as some have reported positive effects of vitamin D on both sleep duration and sleep quality [23,31], whereas others have reported deteriorated sleep quality and increased need of sleep medications [32].

In 2015–2016, a large number of individuals with low serum 25(OH)D (s-25(OH)D) living in the municipality of Tromsø, Northern Norway, were invited to participate in an RCT with vitamin D versus placebo. The primary aim of the study was to investigate the effect of vitamin D supplementation on cardiovascular risk factors. A sleep questionnaire was implemented to provide the opportunity to also study the effect of vitamin D supplementation on sleep. Our hypothesis was that supplementation with vitamin D would increase or normalize sleep duration, reduce daytime sleepiness, and increase recovery from inadequate sleep duration (ISD), excessive daytime sleepiness (EDS) and insomnia.

2. Material and methods

2.1. Population and study design

A detailed description of the study design and results of the main vitamin D intervention study (D-COR) have been published elsewhere [33]. In summary, participants were recruited from the seventh survey of the Tromsø Study (Tromsø7 2015–16), a population-based health study conducted in the municipality of Tromsø, Northern Norway [34]. In Tromsø7, all inhabitants aged 40 years and above (n = 32,591) were invited to participate, of which 21,083 men and women aged 40–99 years attended (65% participation). Measurements of s-25(OH)D were successfully performed in 20,922 participants, of which 1489 participants had s-25(OH)D values < 42 nmol/L and age <80 years and thus were invited by mail to participate in the vitamin D intervention study. In total, 639 individuals were screened by telephone for eligibility. Participants in the main study were included consecutively between June 2015 and December 2016, resulting in 455 participants attending the first visit at the Clinical Research Unit at the University Hospital of North Norway. At the first (fasting) visit, participants were asked to sign an informed consent form prior to clinical examinations, collection of medical history and blood samples. In total, 422 participants were found eligible to participate in the main study, allowing them to attend to the next visit within 2–5 days. The study drugs (Dekristol cholecalciferol capsules (20,000 IU; 500 μg); Mibe GmbH) or identical-looking placebo capsules containing arachis oil (Ayanda GmbH & Co. KG) were distributed during the second (non-fasting) visit, and the participants were instructed to take five capsules as a loading dose followed by one capsule weekly. Also, the participants were asked not to take any vitamin D supplements (including cod liver oil) during the intervention period. After four months the third visit was performed, with examinations identical to the first visit. A total of 411 participants attended the fourth (and final) visit a few days later, at which final examinations were performed and unused study medication was returned and counted. Compliance was calculated as the ratio between capsules used (capsules supplied minus capsules returned) and number of weeks between the second and fourth visits. Adherence to treatment and adverse events have been described in detail elsewhere [33]. In short, the compliance was high (84–100%), no serious study-related adverse events were recorded, and the number and type of side effects did not differ between the groups. All participants and study personnel were blinded throughout the study.

The sleep questionnaire was implemented as part of the first and third visit, about half-way into the inclusion in the main study (from mid-April 2016), with the aim to investigate the effect of vitamin D supplementation on sleep duration and daytime sleepiness, as well as the risk of ISD, EDS and insomnia. In total, 189 participants (92 given vitamin D and 97 given placebo) had both baseline and end of study values for at least one sleep outcome and were included in the present analysis. A flow diagram of the participant inclusion is presented in Fig. 1.

2.2. Measurements

2.2.1. Serum analyses and anthropometric measurements

A detailed description of measurements is published previously [33]. In short, non-fasting serum samples were collected, and measurement of s-25(OH)D (nmol/L) was performed using an in-house liquid chromatography with a tandem mass spectromy (LC-MS/MS) method detecting both s-25(OH)D_3 and s-25(OH)D_2 - the sum of which is presented as s-25(OH)D in the results. The LC-MS/MS analysis was accredited by the Norwegian Accreditation Authority, and the laboratory participates in an international quality surveillance programme, namely the vitamin D external quality assurance scheme (DEQAS), to ensure the analytical reliability of the s-25(OH)D assays. Analyses of serum Calcium (s-Ca) (mmol/L) were done using the Hitachi 917 (Roche Diagnostics), with reagents from Boehringer-Mannheim. Plasma parathyroid hormone (p-PTH) (pmol/L) was measured using an Immulite 2000 Intact PTH analyzer (Siemens Healthcare Diagnostics). Measurements of height and weight were performed with participants wearing light clothing without shoes, and body mass index (BMI) was calculated as weight in kilograms (kg) divided by height in meters squared (m^2). All measurements were performed by trained technicians.

2.2.2. Sleep measures and covariates

The study sleep questionnaire (Supplemental Fig. 1) was a modified version of the sleep questionnaire used in Tromsø7, which has been described in detail elsewhere [35]. In short, the present questionnaire consisted of 18 items, including items to assess sleep duration, the Epworth Sleepiness Scale (ESS) [36] to assess daytime sleepiness, and central items from the Bergen Insomnia Scale (BIS) [37] to assess symptoms of insomnia. Information regarding shift work and sleep medication use was also registered. In the

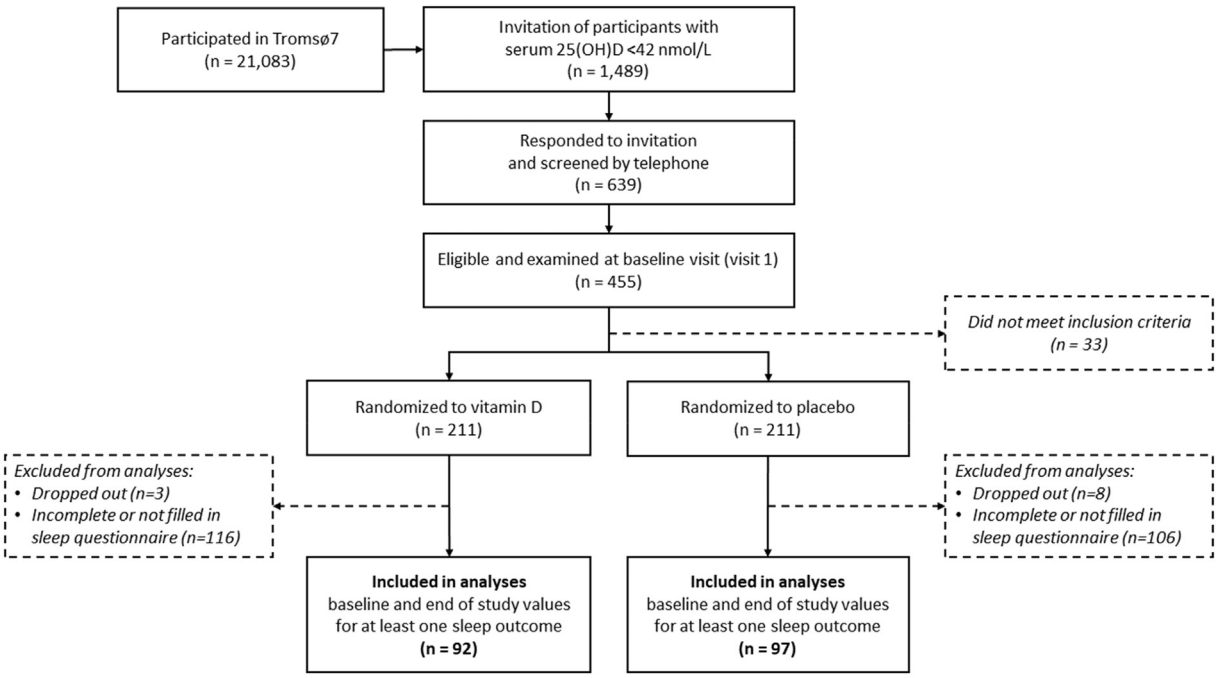


Fig. 1. Flow diagram of the participant inclusion.

following, a detailed description of the sleep items used in the present study is given for each sleep outcome.

To assess sleep duration, the participants were asked to select from pre-specified alternatives which best represented their habitual bedtime, rise time and sleep onset latency (SOL) (ie, the average minutes from bedtime to falling asleep). The participants were asked to report values separately for weekends and weekdays (Supplemental Fig. 1, questions 9–11), in which the latter was used for the present analyses. Sleep duration was calculated by subtracting SOL from time in bed (bedtime subtracted from rise time), and for this purpose the following coding was specified: For participants reporting rise time "before 5 am" or "after 11 am" the value was set to 5 am and 11 am, respectively; bedtime "before 8 pm" or "after 2 am" was set to 8 pm and 2 am, respectively; SOL "more than 60 min" was set to 60 min; and finally, for the SOL alternatives "30–45 min" and "45–60 min", the median value was chosen (37.5 min and 52.5 min, respectively). Sleep duration was dichotomized into ISD (<7 h or ≥9 h) and normal sleep duration (7–9 h), in accordance with the recommendations for adults by the National Sleep Foundation [38].

Daytime sleepiness was assessed using the ESS (Supplemental Fig. 1, question 16), which is a validated tool assessing the probability of a person to fall asleep while engaging certain daily activities expressed as the sum of eight items (ESS-score), scored from 0 (no chance of napping) to 3 (great chance of napping) [36,39]. ESS-scores were then categorized in accordance with Johns et al. [40] into normal daytime sleepiness (ESS-score ≤10) and mild (ESS-score of 11–12), moderate (ESS-score 13–15), and severe EDS (ESS score 16–24). For the present analyses, a dichotomous variable (EDS yes/no) was used, in which EDS was defined as an ESS-score of >10 in accordance with Johns et al. [40].

To assess symptoms of insomnia, we used a slightly modified version of the Bergen Insomnia Scale [37], as described in detail by Sivertsen et al. [35]. In short, the original BIS assessed sleep patterns during the last month, whereas according to the Diagnostic and Statistical Manual for Mental Disorders, Fifth Edition (DSM-5) [41] and the International Classification of Sleep Disorders, Third Edition (ICSD-3) [42] the minimum symptom duration for chronic insomnia is three months. Also, non-restorative sleep is no longer considered an obligate criterion for chronic insomnia. To accommodate these changes, our modified version of the BIS included a question concerning sleep problem duration (Supplemental Fig. 1, question 7), and the BIS item reflecting symptoms of non-restorative sleep (Supplemental Fig. 1, question 4) was disregarded. Thus, in the present study insomnia was defined as being present if the participants reported: 1) at least one of three nocturnal symptoms (prolonged sleep onset, difficulties maintaining sleep and/or early morning awakening) ≥3 nights/week (Supplemental Fig. 1, question 1–3), and 2) one or both of two daytime symptoms (daytime sleepiness/tiredness and/or dissatisfaction with sleep) ≥3 days/week (Supplemental Fig. 1, question 5–6), and 3) a duration of sleep problems for ≥3 months (Supplemental Fig. 1, question 7).

In addition, antidepressant use (including antidepressant medications, mood-stabilizing drugs, and benzodiazepines) was registered, and symptoms of mental distress were assessed using the Beck Depression Inventory II (BDI-II) [43], as described in a previous publication [44].

2.3. Statistical analyses

Statistical analyses were performed using the SPSS software version 27.0 (IBM Corp, Chicago, IL). Normal distribution was evaluated by visual inspection of histograms and Q–Q plots, in combination with evaluation of kurtosis and skewness. Spearman's rho was used to evaluate correlations at baseline. Comparisons of baseline values between groups were performed using the Students *t*-test for continuous variables and the Pearson's χ-square test for categorical variables. Although some deviations from the normality-assumption of the *t*-tests were observed, the results did not change with log-transformation or by running the non-parametric Mann–Whitney U-test.

Baseline and end of study values of s-25(OH)D were compared within the vitamin D and placebo groups using a paired samples t-test.

To assess changes in the categorical sleep outcomes (ISD, EDS, and insomnia), the participants changing from having the outcome of interest at baseline to not having it at the final visit were categorized as "recovered", whereas participants having the outcome at the final visit without having it at the baseline visit were categorized as to have "developed *outcome*". Thereafter, changes between intervention groups were tested using Fisher's exact test, as some of the variables tested showed expected cell count less than 5.

Continuous sleep outcomes (sleep duration and ESS-score) were compared between the vitamin D and placebo groups at the end of the study using a linear regression model with the end of study value as the dependent variable, sex and intervention group as fixed factors, and age and baseline values of the sleep outcome as covariates in accordance with recommendations by Vickers et al. [45].

As previous studies have reported sex-differences in sleep [46], all analyses were performed sex-stratified. Because an effect of vitamin D supplementation was expected to be most likely among participants reporting sleep difficulties (ie, classified with ISD, EDS, and/or insomnia at baseline), we also performed subgroup analyses in these groups. Based on recent recommendations regarding vitamin D deficiency [47], subgroup analyses were also performed according to baseline s-25(OH)D level (above or below 30 nmol/L). Finally, adverse effects on sleep health with repletion of vitamin D were reported during the intervention period [32]. Thus, to allow comparison, we performed subgroup analyses in participants according to s-25(OH)D level at the end of study (above or below 80 nmol/L).

Data are presented as means and standard deviations, unless otherwise specified. Two-sided tests were used for all comparisons, and p-values <0.05 were considered statistically significant.

Power calculations were performed for the main endpoints of the study (ie, cardiovascular risk factors), as previously described [33]. Formal power calculations were not performed for the secondary endpoints regarding sleep.

2.4. Ethics

Written informed consent was obtained from all participants. The D-COR study was approved by the Norwegian Regional Committee for Medical Research Ethics (REK NORD 2013/1464) and by the Norwegian Medicines Agency (2013-003514-40). The study is registered at ClinicalTrials.gov (NCT02750293).

3. Results

In total, 189 participants were included in the present analysis, having both baseline and end of study values for at least one sleep outcome, as illustrated in Fig. 1. Table 1 shows the baseline characteristics for the included study participants. The 222 participants from the main study who were not included in the present analysis did not significantly differ from the included with regards to age, sex, BMI, or smoking status at baseline (data not shown) but had slightly lower mean s-25(OH)D (32.6 ± 12.2 nmol/L *v.* 35.2 ± 12.6 nmol/L, $p = 0.03$).

At baseline, there were no significant differences between the vitamin D and placebo groups (Table 1). Overall, mean age among the participants was 51.5 (range 40–79) years and mean s-25(OH)D was 35.2 (range 12–71) nmol/L. Inclusion in relation to season was similar in the two groups, with most of the participants being included during the winter half-year (Supplemental Fig. 2). There were no significant correlations between sleep duration or ESS-score and age, BMI, s-Ca, p-PTH, or s-25(OH)D at baseline, and neither the risk of ISD, EDS nor insomnia were associated with baseline s-25(OH)D (data not shown).

At the end of the study, after four months, there were no significant differences according to intervention group in sleep duration or ESS-score, nor in the recovery from ISD, EDS or insomnia, both when including all participants, and when analysing men and women separately (Tables 2 and 3).

Subgroup analyses regarding baseline and end of study s-25(OH)D levels and baseline sleep status showed no significant differences between the intervention groups neither in sleep duration, nor in ESS-scores (Supplemental Table 1). Subgroup analyses were not performed for the dichotomous outcomes (ISD, EDS and insomnia) as there were too few events across subgroups.

There was a significant increase in s-25(OH)D at the end of the study of about 51 nmol/L in the vitamin D group ($p < 0.001$) and a significant decrease in the placebo group of about 7 nmol/L ($p < 0.001$). At the end of the study, the p-PTH was significantly lower in the vitamin D group than in the placebo group ($p < 0.001$), whereas there was no difference in s-Ca between the intervention groups (Table 4).

4. Discussion

In the present study we report the results from a secondary analysis of 189 vitamin D insufficient participants in a previously performed RCT. At the end of the study, after four months of treatment, we found no significant effects of vitamin D supplementation on mean sleep duration or mean ESS-score, nor on the recovery from ISD, EDS or insomnia.

Our findings contrast with previous studies on the effect of vitamin D supplementation on sleep health. In a prospective case series from 2013, Huang et al. [23] reported a significant improvement in sleep after 3 months supplementation with vitamin D in 28 US veterans with chronic pain and low s-25(OH)D (<30 ng/mL or <75 nmol/L). In an RCT from 2017, Majid et al. [31] reported that 8 weeks supplementation with 50,000 IU vitamin D significantly increased sleep duration, reduced sleep latency and improved overall subjective sleep quality. However, the sample sizes of these studies were smaller compared to our study, and only one included a proper control group [31]. Nevertheless, the average sleep duration was considerably shorter compared to the present study, in which very few participants reported a sleep duration <5 h. The findings in our study also contrast with another vitamin D intervention study, in which Mason et al. [32] reported that repletion of s-25(OH)D (≥32 ng/mL or 80 nmol/L) among postmenopausal women resulted in an overall worse sleep quality, compared to those who remained insufficient. The results of the present study did not indicate deteriorating effects on sleep duration or ESS-scores, neither from vitamin D supplementation, nor from repletion of s-25(OH)D status (above 80 nmol/L).

The present study has some limitations. The primary aim of the main study was to evaluate the effect of vitamin D supplementation on cardiovascular risk factors. Thus, the inclusion criteria were based on s-25(OH)D measurements in Tromsø7, and not according to specific sleep characteristics. Moreover, the participants were all informed of their vitamin D status through the invitation to participate in the main study, which could potentially have affected their probability of reporting sleep complaints on study visits. Also, most participants were included during winter, which might negatively affect sleep [48,49] and thus could have resulted in higher prevalence of sleep complaints compared to the general population. However, the observed prevalence of sleep complaints in our study was comparable to those previously reported in the Tromsø Study [35] and the importance of seasonal differences in sleep is still a matter of debate [50]. In contrast with previous

Table 1
Baseline characteristics in participants with both baseline and end of study values for at least 1 sleep outcome, by sex and intervention group.

	Overall			Men			Women		
	Vitamin D ($n = 92$)	Placebo ($n = 97$)	p-value	Vitamin D ($n = 47$)	Placebo ($n = 53$)	p-value	Vitamin D ($n = 45$)	Placebo ($n = 44$)	p-value
	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	
Age, years	51.0 (9.0)	52.0 (8.6)	0.475[b]	51.2 (9.6)	51.7 (8.4)	0.755[b]	50.9 (8.4)	52.2 (8.8)	0.474[b]
Sex, % (n)			0.625[a]			–			–
Men	51.5 (47)	54.6 (53)		–	–		–	–	
Women	48.9 (45)	45.4 (44)		–	–		–	–	
Current smokers, % (n)			0.723[a]			0.358[a]			0.635[a]
Yes	19.6 (18)	21.6 (21)		17.0 (8)	24.5 (13)		22.2 (10)	18.2 (8)	
No	80.4 (74)	78.4 (76)		83.0 (39)	75.5 (40)		77.8 (35)	81.8 (36)	
Shift work, % (n)			0.796[a]			0.841[a]			0.973[a]
Yes	14.1 (13)	15.5 (15)		19.1 (9)	20.8 (11)		8.9 (4)	11.4 (5)	
No	85.9 (79)	84.5 (82)		80.9 (38)	79.2 (42)		91.1 (41)	90.9 (39)	
Sleep medication use, % (n)			0.313[a]			0.215[a]			0.699[a]
Yes, now or previously	5.4 (5)	9.3 (9)		2.1 (1)	7.5 (4)		8.9 (4)	11.4 (5)	
No, never used	94.6 (87)	90.7 (88)		97.9 (46)	92.5 (49)		91.1 (41)	88.6 (39)	
BMI, kg/m^2	27.5 (4.2)	27.7 (4.3)	0.696[b]	28.1 (4.3)	28.2 (3.4)	0.942[b]	26.9 (4.2)	27.2 (5.1)	0.708[b]
s-25(OH)D, nmol/L	35.0 (11.8)	35.5 (13.3)	0.775[b]	36.5 (12.9)	36.1 (14.3)	0.880[b]	33.4 (10.4)	34.8 (12.1)	0.556[b]
s-Ca, mmol/L	2.28 (0.07)	2.29 (0.07)	0.286[b]	2.28 (0.07)	2.29 (0.06)	0.813[b]	2.27 (0.06)	2.28 (0.09)	0.248[b]
p-PTH, pmol/L	6.9 (2.1)	6.9 (2.0)	0.958[b]	6.6 (1.9)	6.9 (2.2)	0.414[b]	7.2 (2.3)	6.9 (1.7)	0.464[b]
Sleep duration[c], minutes	412.0 (46.8)	419.8 (57.7)	0.309[b]	407.4 (48.8)	409.5 (53.8)	0.845[b]	416.6 (44.8)	432.6 (60.3)	0.160[b]
Sleep duration[c], % (n)			0.577[a]			0.896[a]			0.337[a]
<5.0 h	2.2 (2)	2.1 (2)		2.2 (1)	3.8 (2)		2.2 (1)	0.0 (0)	
5.0–6.9 h	53.8 (49)	51.0 (49)		58.7 (27)	58.5 (31)		48.9 (22)	41.9 (18)	
7.0–8.9 h	44.0 (40)	44.8 (43)		39.1 (18)	37.7 (20)		48.9 (22)	53.5 (23)	
≥9.0 h	0.0 (0)	2.1 (2)		0.0 (0)	0.0 (0)		0.0 (0)	4.7 (2)	
ISD[c], % (n)			0.908[a]			0.887[a]			0.666[a]
Yes (<7 or ≥9 h)	56.0 (51)	55.2 (53)		60.9 (28)	62.3 (33)		51.1 (23)	46.5 (20)	
No	44.0 (40)	44.8 (43)		39.1 (18)	37.7 (20)		48.9 (22)	53.5 (23)	
ESS-score[d]	5.9 (3.0)	5.7 (2.9)	0.668[b]	5.8 (2.6)	5.8 (3.0)	0.948[b]	5.9 (3.4)	5.5 (2.8)	0.589[b]
ESS-score[d], % (n)			0.058[a]			0.181[a]			0.310[a]
0–10 [Normal]	94.5 (86)	92.8 (90)		195.7 (45)	90.6 (48)		93.2 (41)	95.5 (42)	
11–12 [Mild EDS]	2.2 (2)	7.2 (7)		2.1 (1)	9.4 (5)		2.3 (1)	4.5 (2)	
13–15 [Moderate EDS]	3.3 (3)	0.0 (0)		2.1 (1)	0.0 (0)		4.5 (2)	0.0 (0)	
16–24 [Severe EDS]	0.0 (0)	0.0 (0)		0.0 (0)	0.0 (0)		0.0 (0)	0.0 (0)	
EDS[d], % (n)			0.629[a]			0.311[a]			0.645[a]
Yes	5.5 (5)	7.2 (7)		4.3 (2)	9.4 (5)		6.8 (3)	4.5 (2)	
No	94.5 (86)	92.8 (90)		95.7 (45)	90.6 (48)		93.2 (41)	95.5 (42)	
Insomnia[e], % (n)			0.892[a]			0.967[a]			0.814[a]
Yes	18.0 (16)	18.8 (18)		19.6 (9)	19.2 (10)		16.3 (7)	18.2 (8)	
No	82.0 (73)	81.3 (78)		80.4 (37)	80.8 (42)		83.7 (36)	81.8 (36)	
BDI-II score	5.0 (4.8)	5.1 (5.4)	0.857[b]	5.0 (4.4)	3.9 (3.9)	0.208[b]	5.0 (5.3)	6.5 (6.7)	0.220[b]
BDI-II score, % (n)			0.319[a]			0.506[a]			0.163[a]
0–13 (minimal)	94.5 (86)	88.7 (86)		95.7 (44)	96.2 (51)		93.3 (42)	79.5 (35)	
14–19 (mild)	3.3 (3)	8.2 (8)		2.2 (1)	3.8 (2)		4.4 (2)	13.6 (6)	
20–28 (moderate)	2.2 (2)	3.1 (3)		2.2 (1)	0 (0)		2.2 (1)	6.8 (3)	
≥29 (severe)[f]	–	–		–	–		–	–	
Antidepressant use, % (n)			0.462[a]			0.488[a]			0.717[a]
Yes, now or previously	6.5 (6)	4.1 (4)		4.3 (2)	1.9 (1)		8.9 (4)	6.8 (3)	
No, never used	93.5 (86)	95.9 (93)		95.7 (45)	98.1 (52)		91.1 (41)	93.2 (41)	

Abbreviations: n = number of participants; SD = standard deviation; BMI = body mass index; s-Ca = serum calcium; p-PTH = plasma parathyroid hormone; s-25(OH)D = serum 25-hydroxyvitamin D; ISD = inadequate sleep duration; ESS = epworth sleepiness scale; EDS = excessive daytime sleepiness; BDI-II = beck depression inventory II.
Values represent means (SD) if not otherwise specified. Each characteristic was compared between the vitamin D and the placebo group.
P-values <0.05 were considered significant.

[a] Pearson's χ-square for categorical variables.
[b] Student's t-test for continuous variables.
[c] Baseline values available in (n = 91) participants in the vitamin D group, and (n = 96) in the placebo group.
[d] Baseline values available in (n = 91) participants in the vitamin D group, and (n = 97) in the placebo group.
[e] Baseline values available in (n = 89) participants in the vitamin D group, and (n = 96) in the placebo group.
[f] Not eligible for participation in the study.

intervention studies [23,31,32], our study did not use the Pittsburgh Sleep Quality Index (PSQI) [51]. It cannot be excluded that additional information from the PSQI (eg, afflictions of night sweat and/or pain) could have changed the results of the present study. Also, additional medical conditions and/or use of medications (other than sleep medications and antidepressants) with the potential to affect the sleep/wake cycle were not specifically asked for, neither was cognitive behavioral therapy or other treatments of sleep complaints. However, there were no between-groups differences regarding the assessed covariates at baseline (as shown in Table 1), indicating a successful randomization procedure. Thus, potential effects of unmeasured factors were most likely accommodated by randomly distributing them between intervention groups. Also, we cannot exclude that our lack of findings was due to the choice of sleep variables. In particular, we did not have objective sleep measures to complement the subjective sleep outcome analyses. Moreover, the study duration was relatively short. Because the study enrolled participants with low vitamin D levels, four months were considered long enough to demonstrate an effect on the main study endpoints, but also short enough to ethically

Table 2
Change of status regarding ISD, EDS and insomnia after four months intervention with vitamin D or placebo.

	All participants ($N = 189$)			Men ($n = 100$)			Women ($n = 89$)		
	Vitamin D	Placebo	*p*-value	Vitamin D	Placebo	*p*-value	Vitamin D	Placebo	*p*-value
	% (*n*)	% (*n*)		% (*n*)	% (*n*)		% (*n*)	% (*n*)	
ISD ($n = 185$)			0.558			0.638			1.000
Recovered	20.2 (18)	11.5 (11)		21.7 (10)	7.5 (4)		18.6 (8)	16.3 (7)	
Developed ISD	11.2 (10)	10.4 (10)		8.7 (4)	5.7 (3)		14.0 (6)	16.3 (7)	
No change	68.5 (61)	78.1 (75)		69.6 (32)	86.8 (46)		67.4 (29)	67.4 (29)	
EDS ($n = 186$)			1.000			0.333			1.000
Recovered	2.2 (2)	3.1 (3)		0.0 (0)	3.8 (2)		4.5 (2)	2.3 (1)	
Developed EDS	2.2 (2)	1.0 (1)		2.2 (1)	0.0 (0)		2.3 (1)	2.3 (1)	
No change	95.6 (86)	95.8 (92)		97.8 (45)	96.2 (50)		93.2 (41)	95.5 (42)	
Insomnia ($n = 179$)			1.000			1.000			1.000
Recovered	6.0 (5)	5.2 (5)		7.1 (3)	5.8 (3)		4.9 (2)	4.5 (2)	
Developed insomnia	3.6 (3)	3.1 (3)		0.0 (0)	1.9 (1)		7.3 (3)	4.5 (2)	
No change	90.4 (75)	91.7 (88)		92.9 (39)	92.3 (48)		87.8 (36)	90.9 (40)	

Abbreviations: n = number of participants; ISD = inadequate sleep duration; EDS = excessive daytime sleepiness. Recovery versus development of each outcome was compared between the vitamin D and the placebo group using Fisher's exact test. *P*-values <0.05 were considered significant.

Table 3
Change in sleep duration and ESS-score after four months intervention with vitamin D or placebo.

	Sleep duration[c] (minutes)						ESS-score[d]					
	Vitamin D		Placebo		Difference (95% CI)	*p*-value	Vitamin D		Placebo		Difference (95% CI)	*p*-value
	n	Mean (SD)	*n*	Mean (SD)			*n*	Mean (SD)	*n*	Mean (SD)		
Overall	89		96				90		96			
Baseline		412.3 (47.2)		419.8 (57.7)	−7.5 (−22.9, 7.8)			5.8 (2.9)		5.6 (2.9)	0.2 (−0.7, 1.0)	
End of study		420.1 (55.0)		419.7 (60.3)	0.4 (−16.3, 17.2)			5.5 (2.9)		5.6 (2.9)	−0.1 (−0.9, 0.8)	
Delta		7.8 (33.4)		−0.1 (40.6)	7.9 (−2.9, 18.8)	0.149		−0.3 (1.8)		−0.1 (2.0)	−0.2 (−0.8, 0.3)	0.421
ANCOVA		.		.	6.9 (−3.6, 17.4)	0.196[a]		.		.	−0.2 (−0.7, 0.3)	0.511[a]
Men	46		53				46		52			
Baseline		407.4 (48.8)		409.5 (53.8)	−2.0 (−22.6, 18.6)			5.7 (2.4)		5.7 (3.0)	−0.0 (−1.1, 1.1)	
End of study		416.1 (61.8)		406.3 (57.7)	9.8 (−14.0, 33.7)			5.5 (2.7)		6.2 (2.9)	−0.7 (−1.8, 0.4)	
Delta		8.7 (34.6)		−3.2 (41.4)	11.8 (−3.5, 27.2)	0.129		−0.2 (1.7)		0.5 (2.1)	−0.7 (−1.5, 0.1)	0.089
ANCOVA		.		.	11.9 (−3.3, 27.1)	0.125[b]		.		.	−0.7 (−1.4, 0.1)	0.071[b]
Women	43		43				44		44			
Baseline		417.5 (45.5)		432.6 (60.3)	−15.0 (−38.0, 7.8)			5.9 (3.4)		5.5 (2.8)	0.4 (−1.0, 1.7)	
End of study		424.4 (46.9)		436.2 (60.0)	−11.8 (−34.9, 11.3)			5.5 (3.1)		4.8 (2.7)	0.7 (−0.5, 1.9)	
Delta		6.9 (32.3)		3.7 (39.7)	3.2 (−12.2, 18.8)	0.678		−0.4 (1.9)		−0.7 (1.6)	0.3 (−0.4, 1.1)	0.405
ANCOVA		.		.	0.6 (−14.1, 15.2)	0.936[b]		.		.	0.4 (−0.3, 1.1)	0.253[b]

Abbreviations: n = number of participants; ESS = epworth sleepiness scale; SD = standard deviation; CI = confidence interval; ANCOVA = analysis of covariance.
Values represent means (SD) if not otherwise specified, and the significance level was set to $p < 0.05$ for all comparisons. Delta values (end of study value − baseline value) were compared between the vitamin D and the placebo group using the Students *t*-test. The ANCOVA analyses were done comparing end of study values in the vitamin D v. placebo group using linear regression.
[a] Baseline values, age, and sex as covariates.
[b] Baseline values and age as covariates.
[c] 185 participants had valid records of both baseline and last visit values and were included in the analyses.
[d] 186 participants had valid records of both baseline and last visit values and were included in the analyses.

Table 4
Baseline and end of study values in participants with records of both baseline and end of study values for at least 1 sleep outcome, by sex and intervention group.

	Overall			Men			Women		
	Vitamin D ($n = 92$)	Placebo ($n = 97$)	*p*-value	Vitamin D ($n = 47$)	Placebo ($n = 53$)	*p*-value	Vitamin D ($n = 45$)	Placebo ($n = 44$)	*p*-value
	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	
BMI_{BL}, kg/m^2	27.5 (4.2)	27.7 (4.3)		28.1 (4.3)	28.2 (3.4)		26.9 (4.2)	27.2 (5.1)	
BMI_{END}, kg/m^2	27.8 (4.2)	28.0 (4.4)		28.3 (4.3)	28.4 (3.6)		27.2 (4.2)	27.5 (5.3)	
Δ BMI, kg/m^2	0.3 (0.6)	0.3 (0.6)	0.925	0.23 (0.7)	0.20 (0.5)	0.815	0.28 (0.5)	0.31 (0.6)	0.856
s-Ca_{BL}, mmol/L	2.28 (0.07)	2.29 (0.07)		2.28 (0.07)	2.29 (0.06)		2.27 (0.06)	2.28 (0.09)	
s-Ca_{END}, mmol/L	2.28 (0.07)	2.27 (0.08)		2.28 (0.06)	2.28 (0.07)		2.28 (0.08)	2.26 (0.09)	
Δ s-Ca, mmol/L	0.00 (0.07)	−0.01 (0.07)	0.057	0.00 (0.05)	−0.01 (0.06)	0.597	0.01 (0.08)	−0.02 (0.08)	0.050
p-PTH_{BL}, pmol/L	6.9 (2.1)	6.9 (2.0)		6.6 (1.9)	6.9 (2.2)		7.2 (2.3)	6.9 (1.7)	
p-PTH_{END}, pmol/L	6.2 (1.8)	7.8 (2.4)		6.1 (1.7)	7.6 (2.6)		6.4 (1.9)	7.9 (2.0)	
Δ p-PTH, pmol/L	−0.7 (1.6)	0.8 (1.5)	<0.001*	−0.5 (1.3)	0.7 (1.7)	<0.001*	−0.8 (1.8)	1.0 (1.3)	<0.001*
s-25(OH)D_{BL}, nmol/L	35.0 (11.8)	35.5 (13.3)		36.5 (12.9)	36.1 (14.3)		33.4 (10.4)	34.8 (12.1)	
s-25(OH)D_{END}, nmol/L	85.9 (19.8)	28.6 (8.9)		85.2 (21.9)	29.1 (9.2)		86.5 (17.5)	28.1 (8.7)	
Δ s-25(OH)D, nmol/L	50.9 (22.4)	−6.9 (10.9)	<0.001*	48.7 (24.6)	−7.0 (11.2)	<0.001*	53.1 (19.9)	−6.8 (10.66)	<0.001*

Abbreviations: n = number of participants; SD = standard deviation; BL = baseline; END = end of study; BMI = body mass index; s-Ca = serum calcium; p-PTH = plasma parathyroid hormone; s-25(OH)D = serum 25-hydroxyvitamin D. Values represent means (SD) if not otherwise specified. Delta values are presented as Δ and calculated as (end of study value − baseline value). Each characteristic was compared between the vitamin D and the placebo group using Student's t-test. *P*-values <0.05 were considered significant and are denoted *.

defend postponing vitamin D supplementation of participants with known vitamin D deficiency randomized to placebo. A longer intervention period might have been needed to show an effect of vitamin D, although notably the intervention period was considerably shorter in the study by Majid et al. [31]. Finally, the sample size of the present study was relatively small and separate power-calculations regarding the secondary sleep outcomes were not performed.

The present study also has strengths, including the use of a gold-standard RCT-study design with strict inclusion criteria, including only vitamin D insufficient participants at baseline. Moreover, the questionnaire was based on previously validated sleep instruments used in Tromsø7, albeit with minor modifications. Finally, the vitamin D dosing regimen significantly increased s-25(OH)D in the vitamin D group, and both adherence to treatment and compliance were exceptionally high.

5. Conclusion

In conclusion, we were not able to demonstrate any significant effect of vitamin D supplementation on various sleep outcomes in this vitamin D insufficient population.

Funding

The main study was supported by grants from the North Norway Regional Health Authorities (grant number SFP1277-16) and UiT The Arctic University of Norway. AUL received funding through the PhD Program at UiT The Arctic University of Norway. The publication charges for this article were funded through UiT The Arctic University of Norway's Publishing Agreements. The funding body did not have a role in the design of the study, data collection, analysis, interpretation of data or writing of the manuscript.

Contribution by the authors

AUL formulated the research questions with important input from GG, LAH and RJ. RJ was responsible for data collection and design of the study. RJ was responsible for applications of formal and ethical approvals through the Tromsø Study and REK. AUL drafted the manuscript and conducted the statistical analyses. LAH, RJ and GG critically revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

Credit author statement

Larsen, AU: Conceptualization, Methodology, Data curation, Formal analysis, Writing – Original draft, Visualization. **Hopstock, L:** Writing – Review & Editing. **Jorde, R:** Investigation, Resources, Data curation, Writing – Review & Editing, Project administration, Funding acquisition. **Grimnes, G:** Methodology, Writing – Review & Editing, Supervision.

Acknowledgements

The authors would like to thank all participants that attended the D-COR Study, and the staff at the Centre for Clinical Research and Education, the University Hospital of North Norway. The authors also gratefully acknowledge the contributions made by Børge Sivertsen providing the syntax for the insomnia variable.

Conflict of interest

The authors have no competing interests to declare.

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: https://doi.org/10.1016/j.sleepx.2021.100040

References

[1] National Institute of Mental Health. Arousal and regulatory systems: workshop proceedings. 09.06.2021 from, https://www.nimh.nih.gov/research/research-funded-by-nimh/rdoc/arousal-and-regulatory-systems-workshop-proceedings; 2013.
[2] Dalmases M, Benítez I, Sapiña-Beltran E, et al. Impact of sleep health on self-perceived health status. Sci Rep 2019;9(1):7284. https://doi.org/10.1038/s41598-019-43873-5.
[3] Cedernaes J, Schiöth HB, Benedict C. Determinants of shortened, disrupted, and mistimed sleep and associated metabolic health consequences in healthy humans. Diabetes 2015;64(4):1073–80. https://doi.org/10.2337/db14-1475.
[4] Cappuccio FP, D'Elia L, Strazzullo P, et al. Quantity and quality of sleep and incidence of type 2 diabetes: a systematic review and meta-analysis. Diabetes Care 2010;33(2):414–20. https://doi.org/10.2337/dc09-1124.
[5] Harvey AG. Sleep and circadian functioning: critical mechanisms in the mood disorders? Annu Rev Clin Psychol 2011;7:297–319. https://doi.org/10.1146/annurev-clinpsy-032210-104550.
[6] Huyett P, Siegel N, Bhattacharyya N. Prevalence of sleep disorders and association with mortality: results from the NHANES 2009-2010. Laryngoscope 2021;131(3):686–9. https://doi.org/10.1002/lary.28900.
[7] Hillman D, Mitchell S, Streatfeild J, et al. The economic cost of inadequate sleep. Sleep 2018;41(8). https://doi.org/10.1093/sleep/zsy083.
[8] Gominak SC, Stumpf WE. The world epidemic of sleep disorders is linked to vitamin D deficiency. Med Hypotheses 2012;79(2):132–5. https://doi.org/10.1016/j.mehy.2012.03.031.
[9] Yan S, Tian Z, Zhao H, et al. A meta-analysis: does vitamin D play a promising role in sleep disorders? Food Sci Nutr 2020;8(10):5696–709. https://doi.org/10.1002/fsn3.1867.
[10] Romano F, Muscogiuri G, Di Benedetto E, et al. Vitamin D and sleep regulation: is there a role for vitamin D? Curr Pharmaceut Des 2020;26(21):2492–6. https://doi.org/10.2174/1381612826666200310145935.
[11] Muscogiuri G, Barrea L, Scannapieco M, et al. The lullaby of the sun: the role of vitamin D in sleep disturbance. Sleep Med 2019;54:262–5. https://doi.org/10.1016/j.sleep.2018.10.033.
[12] DeLuca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr 2004;80(6 Suppl):1689s. https://doi.org/10.1093/ajcn/80.6.1689S. 96s.
[13] Eyles DW, Smith S, Kinobe R, et al. Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain. J Chem Neuroanat 2005;29(1):21–30. https://doi.org/10.1016/j.jchemneu.2004.08.006.
[14] Garcion E, Wion-Barbot N, Montero-Menei CN, et al. New clues about vitamin D functions in the nervous system. Trends Endocrinol Metabol 2002;13(3):100–5. https://doi.org/10.1016/s1043-2760(01)00547-1.
[15] Pardridge WM, Sakiyama R, Coty WA. Restricted transport of vitamin D and A derivatives through the rat blood-brain barrier. J Neurochem 1985;44(4):1138–41. https://doi.org/10.1111/j.1471-4159.1985.tb08735.x.
[16] Gutierrez-Monreal MA, Cuevas-Diaz Duran R, Moreno-Cuevas JE, et al. A role for 1α,25-dihydroxyvitamin d3 in the expression of circadian genes. J Biol Rhythm 2014;29(5):384–8. https://doi.org/10.1177/0748730414549239.
[17] Masood T, Kushwaha RS, Singh R, et al. Circadian rhythm of serum 25 (OH) vitamin D, calcium and phosphorus levels in the treatment and management of type-2 diabetic patients. Drug Discov Ther 2015;9(1):70–4. https://doi.org/10.5582/ddt.2015.01002.
[18] Lucock M, Jones P, Martin C, et al. Vitamin D: beyond metabolism. J Evid Based Complementary Altern Med 2015;20(4):310–22. https://doi.org/10.1177/2156587215580491.
[19] Kaneko I, Sabir MS, Dussik CM, et al. 1,25-Dihydroxyvitamin D regulates expression of the tryptophan hydroxylase 2 and leptin genes: implication for behavioral influences of vitamin D. Faseb J 2015;29(9):4023–35. https://doi.org/10.1096/fj.14-269811.
[20] Amaral FGD, Cipolla-Neto J. A brief review about melatonin, a pineal hormone. Arch Endocrinol Metab 2018;62(4):472–9. https://doi.org/10.20945/2359-3997000000066.
[21] Shipton EA, Shipton EE. Vitamin D and pain: vitamin D and its role in the aetiology and maintenance of chronic pain states and associated comorbidities. Pain Res Treat 2015;2015:904967. https://doi.org/10.1155/2015/904967.
[22] de Oliveira DL, Hirotsu C, Tufik S, et al. The interfaces between vitamin D, sleep and pain. J Endocrinol 2017;234(1):R23–36. https://doi.org/10.1530/JOE-16-0514.
[23] Huang W, Shah S, Long Q, et al. Improvement of pain, sleep, and quality of life in chronic pain patients with vitamin D supplementation. Clin J Pain 2013;29(4):341–7. https://doi.org/10.1097/AJP.0b013e318255655d.
[24] Kim JH, Chang JH, Kim DY, et al. Association between self-reported sleep duration and serum vitamin D level in elderly Korean adults. J Am Geriatr Soc 2014;62(12):2327–32. https://doi.org/10.1111/jgs.13148.
[25] Massa J, Stone KL, Wei EK, et al. Vitamin D and actigraphic sleep outcomes in older community-dwelling men: the MrOS sleep study. Sleep 2015;38(2):251–7. https://doi.org/10.5665/sleep.4408.

[26] Liu X, Ke L, Ho J, et al. Sleep duration is associated with vitamin D deficiency in older women living in Macao, China: a pilot cross-sectional study. PLoS One 2020;15(3):e0229642. https://doi.org/10.1371/journal.pone.0229642.
[27] McCarty DE, Reddy A, Keigley Q, et al. Vitamin D, race, and excessive daytime sleepiness. J Clin Sleep Med 2012;8(6):693–7. https://doi.org/10.5664/jcsm.2266.
[28] Beydoun MA, Gamaldo AA, Canas JA, et al. Serum nutritional biomarkers and their associations with sleep among US adults in recent national surveys. PLoS One 2014;9(8):e103490. https://doi.org/10.1371/journal.pone.0103490.
[29] Nakamura K, Hui SP, Ukawa S, et al. Serum 25-hydroxyvitamin D(3) levels and poor sleep quality in a Japanese population: the DOSANCO Health Study. Sleep Med 2019;57:135–40. https://doi.org/10.1016/j.sleep.2019.01.046.
[30] Jung YS, Chae CH, Kim YO, et al. The relationship between serum vitamin D levels and sleep quality in fixed day indoor field workers in the electronics manufacturing industry in Korea. Ann Occup Environ Med 2017;29:25. https://doi.org/10.1186/s40557-017-0187-7z.
[31] Majid MS, Ahmad HS, Bizhan H, et al. The effect of vitamin D supplement on the score and quality of sleep in 20-50 year-old people with sleep disorders compared with control group. Nutr Neurosci 2018;21(7):511–9. https://doi.org/10.1080/1028415X.2017.1317395.
[32] Mason C, de Dieu Tapsoba J, Duggan C, et al. Repletion of vitamin D associated with deterioration of sleep quality among postmenopausal women. Prev Med 2016;93:166–70. https://doi.org/10.1016/j.ypmed.2016.09.035.
[33] Kubiak J, Thorsby PM, Kamycheva E, et al. Vitamin D supplementation does not improve CVD risk factors in vitamin D-insufficient subjects. Endocr Connect 2018;7(6):840–9. https://doi.org/10.1530/EC-18-0144.
[34] Jacobsen BK, Eggen AE, Mathiesen EB, et al. Cohort profile: the tromso study. Int J Epidemiol 2012;41(4):961–7. https://doi.org/10.1093/ije/dyr049.
[35] Sivertsen B, Pallesen S, Friborg O, et al. Sleep patterns and insomnia in a large population-based study of middle-aged and older adults: the Tromsø study 2015-2016. J Sleep Res 2021;30(1):e13095. https://doi.org/10.1111/jsr.13095.
[36] Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 1991;14(6):540–5. https://doi.org/10.1093/sleep/14.6.540.
[37] Pallesen S, Bjorvatn B, Nordhus IH, et al. A new scale for measuring insomnia: the Bergen Insomnia Scale. Percept Mot Skills 2008;107(3):691–706. https://doi.org/10.2466/pms.107.3.691-706.
[38] Hirshkowitz M, Whiton K, Albert SM, et al. National Sleep Foundation's updated sleep duration recommendations: final report. Sleep Health 2015;1(4):233–43. https://doi.org/10.1016/j.sleh.2015.10.004.
[39] Lapin BR, Bena JF, Walia HK, et al. The epworth sleepiness scale: validation of one-dimensional factor structure in a large clinical sample. J Clin Sleep Med 2018;14(8):1293–301. https://doi.org/10.5664/jcsm.7258.
[40] Johns MW. The official website of the epworth sleepiness scale (ESS) and the epworth sleepiness scale for children and adolecents (ESS-CHADS). 09.06. 2021 from, http://www.epworthsleepinessscale.com/; 2016.
[41] American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5th ed. Washington, DC: American Psychiatric Publishing; 2013. https://doi.org/10.1176/appi.books.9780890425596.
[42] Sateia MJ. International classification of sleep disorders-third edition: highlights and modifications. Chest 2014;146(5):1387–94. https://doi.org/10.1378/chest.14-0970.
[43] Steer RA, Clark DA, Beck AT, et al. Common and specific dimensions of self-reported anxiety and depression: the BDI-II versus the BDI-IA. Behav Res Ther 1999;37(2):183–90. https://doi.org/10.1016/s0005-7967(98)00087-4.
[44] Jorde R, Kubiak J. No improvement in depressive symptoms by vitamin D supplementation: results from a randomised controlled trial. J Nutr Sci 2018;7:e30. https://doi.org/10.1017/jns.2018.19.
[45] Vickers AJ, Altman DG. Statistics notes: analysing controlled trials with baseline and follow up measurements. BMJ 2001;323(7321):1123–4. https://doi.org/10.1136/bmj.323.7321.1123.
[46] Mong JA, Cusmano DM. Sex differences in sleep: impact of biological sex and sex steroids. Philos Trans R Soc Lond B Biol Sci 2016;371(1688):20150110. https://doi.org/10.1098/rstb.2015.0110.
[47] Amrein K, Scherkl M, Hoffmann M, et al. Vitamin D deficiency 2.0: an update on the current status worldwide. Eur J Clin Nutr 2020;74(11):1498–513. https://doi.org/10.1038/s41430-020-0558-y.
[48] Lowden A, Lemos NAM, Gonçalves BSB, et al. Delayed sleep in winter related to natural daylight exposure among arctic day workers. Clocks Sleep 2019;1(1):105–16. https://doi.org/10.3390/clockssleep1010010.
[49] Putilov AA. Retrospectively reported month-to-month variation in sleeping problems of people naturally exposed to high-amplitude annual variation in daylength and/or temperature. Sleep Sci 2017;10(3):101–12. https://doi.org/10.5935/1984-0063.20170019.
[50] Sivertsen B, Friborg O, Pallesen S, et al. Sleep in the land of the midnight sun and polar night: the Tromsø study. Chronobiol Int 2020;38(33):334–42. https://doi.org/10.1080/07420528.2020.1845191.
[51] Buysse DJ, Reynolds 3rd CF, Monk TH, et al. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatr Res 1989;28(2):193–213. https://doi.org/10.1016/0165-1781(89)90047-4.

Supplemental Table 1. Change in sleep duration and ESS-score after four months intervention with vitamin D or placebo in relation to final and baseline s-25(OH)D and sleep status at baseline[1].

	Δ Sleep duration (minutes)						Δ ESS-score					
	Vitamin D		Placebo		Adj. difference (95% CI)	*p*-value	Vitamin D		Placebo		Adj. difference (95% CI)	*p*-value
	n	Mean (SD)	*n*	Mean (SD)			*n*	Mean (SD)	*n*	Mean (SD)		
Baseline s- 25(OH)D												
≥30 nmol/L	55	4.0 (31.9)	96	-0.1 (40.6)	2.1 (-9.8, 14.2)	.722	56	-0.4 (1.8)	96	-0.1 (2.0)	-0.3 (-0.9, 0.3)	.349
<30 nmol/L	34	14.0 (35.2)	96	-0.1 (40.6)	13.1 (-2.2, 28.4)	.094	34	-0.1 (1.8)	96	-0.1 (2.0)	-0.0 (-0.8, 0.7)	.915
Final s- 25(OH)D												
>80 nmol/L	56	5.0 (35.3)	96	-0.1 (40.6)	3.4 (-9.0, 15.8)	.586	55	-0.3 (1.8)	96	-0.1 (2.0)	-0.2 (-0.8, 0.4)	.448
≤80 nmol/L	33	12.6 (29.8)	96	-0.1 (40.6)	12.0 (-2.8, 26.8)	.111	35	-0.2 (1.9)	96	-0.1 (2.0)	-0.0 (-0.7, 0.6)	.907
ISD at baseline												
Yes	49	15.0 (30.7)	53	6.7 (39.1)	7.7 (-6.2, 21.7)	.274	49	-0.0 (1.9)	52	0.1 (2.1)	-0.2 (-0.9, 0.6)	.668
No	40	-0.9 (34.8)	43	-8.5 (41.1)	5.9 (-10.7, 22.6)	.480	40	-0.6 (1.6)	43	-0.3 (1.9)	-0.2 (-0.9, 0.5)	.609
EDS at baseline												
Yes	5	-24.5 (56.6)	7	4.6 (21.2)	-41.8 (-83.5, -0.1)	.050	4	-1.8 (1.7)	7	-0.4 (2.3)	0.4 (-3.9, 4.7)	.835
No	83	9.9 (31.1)	89	-0.5 (41.8)	9,4 (-1.4, 20.2)	.088	86	-0.2 (1.8)	89	-0.0 (2.0)	-0.1 (-0.6, 0.4)	.692
Insomnia at baseline												
Yes	15	10.2 (43.3)	18	12.4 (48.5)	2.1 (-29.1, 33.3)	.891	16	0.2 (2.2)	18	0.3 (1.8)	-0.3 (-1.5, 1.0)	.638
No	71	7.9 (31.7)	77	-2.6 (38.4)	8.7 (-2.8, 20.3)	.135	71	-0.4 (1.8)	78	-0.1 (2.0)	-0.2 (-0.8, 0.4)	.505

Abbreviations: SD = standard deviation; CI = confidence interval; s-25(OH)D = serum 25-hydroxyvitamin D; ISD = inadequate sleep duration; EDS = excessive daytime sleepiness. Delta (Δ) values (end of study value – baseline value) are presented as means (SD).

[1] End of study values in the vitamin D *v.* placebo group analysed with linear regression with baseline values, age, and sex as covariates. *P*-values <0.05 were considered significant.

D-COR Sleep Filled in at ☐ visit 1 ☐ visit 3 Number: ☐☐☐☐

Questions about sleep

Initials: ☐☐☐☐

Date: ☐☐.☐☐.☐☐☐☐

How many days a week?

(Tick the number of days)

		No days	**1 day**	**2 days**	**3 days**	**4 days**	**5 days**	**6 days**	**7 days**
1.	Do you usually use more than 30 minutes to fall asleep after the light was switched off?	☐	☐	☐	☐	☐	☐	☐	☐
2.	Do you usually wake up for more than 30 minutes in the middle of the night?	☐	☐	☐	☐	☐	☐	☐	☐
3.	Do you wake up 30 minutes earlier than you wished, without being able to go back asleep again?	☐	☐	☐	☐	☐	☐	☐	☐
4.	Have you not felt refreshed after sleep?	☐	☐	☐	☐	☐	☐	☐	☐
5.	Felt so tired that it has affected your work, school or private life?	☐	☐	☐	☐	☐	☐	☐	☐
6.	Have you been dissatisfied with your sleep?	☐	☐	☐	☐	☐	☐	☐	☐

7. If sleep problems, how long time?

- ☐ Less than 1 week
- ☐ 1 - 3 weeks
- ☐ 1 month
- ☐ 2 months
- ☐ 3 months
- ☐ 4 - 6 months
- ☐ 7 - 12 months
- ☐ More than 1 year
- ☐ Do not have sleep problems

8. Do you usually work shifts or at night?

☐ No ☐ Yes

9. When do you usually go to sleep?

On work days	**On non-working days**
☐ Before 20:00 PM	☐ Before 20:00 PM
☐ 20:00	☐ 20:00
☐ 20:30	☐ 20:30
☐ 21:00	☐ 21:00
☐ 21:30	☐ 21:30
☐ 22:00	☐ 22:00
☐ 22:30	☐ 22:30
☐ 23:00	☐ 23:00
☐ 23:30	☐ 23:30
☐ 00:00	☐ 00:00
☐ 00:30	☐ 00:30
☐ 01:00	☐ 01:00
☐ 01:30	☐ 01:30
☐ 02:00	☐ 02:00
☐ After 02:00 AM	☐ After 02:00 AM

10. For how long are you awake before you fall asleep?

On work days	**On non-working days**
☐ Immediately	☐ Immediately
☐ After 5 minutes	☐ After 5 minutes
☐ After 10 minutes	☐ After 10 minutes
☐ After 15 minutes	☐ After 15 minutes
☐ After 20 minutes	☐ After 20 minutes
☐ After 25 minutes	☐ After 25 minutes
☐ After 30 minutes	☐ After 30 minutes
☐ After 30-35 minutes	☐ After 30-35 minutes
☐ After 45-60 minutes	☐ After 45-60 minutes
☐ After more than 60 minutes	☐ After more than 60 minutes

11. What time do you usually wake up?

On work days	**On non-working days**
☐ Before 05:00 AM	☐ Before 05:00 AM
☐ 05:00	☐ 05:00
☐ 05:30	☐ 05:30
☐ 06:00	☐ 06:00
☐ 06:30	☐ 06:30
☐ 07:00	☐ 07:00
☐ 07:30	☐ 07:30
☐ 08:00	☐ 08:00
☐ 08:30	☐ 08:30
☐ 09:00	☐ 09:00
☐ 09:30	☐ 09:30
☐ 10:00	☐ 10:00
☐ 10:30	☐ 10:30
☐ 11:00	☐ 11:00
☐ After 11:00 AM	☐ After 11:00 AM

12. How often do you take naps at daytime?

- ☐ Never or less than once per month
- ☐ Less than once per week
- ☐ 1-2 days per week
- ☐ 3-5 days per week
- ☐ Daily or almost daily

13. If you take a nap, how long does it usually last?

- ☐ 5 minutes
- ☐ 5-15 minutes
- ☐ 15-30 minutes
- ☐ 30-45 minutes
- ☐ 1 hour – 1 ½ hours
- ☐ 1 ½ hours – 2 hours
- ☐ 2 hours – 2 ½ hours
- ☐ 2 ½ hours – 3 hours
- ☐ More than 3 hours

14. Do you snore while sleeping?

- ☐ Never or less than one night per month
- ☐ Less than one night per week
- ☐ 1-2 nights per week
- ☐ 3-5 nights per week
- ☐ Daily or almost daily

15. Have you had breating pauses when you sleep?

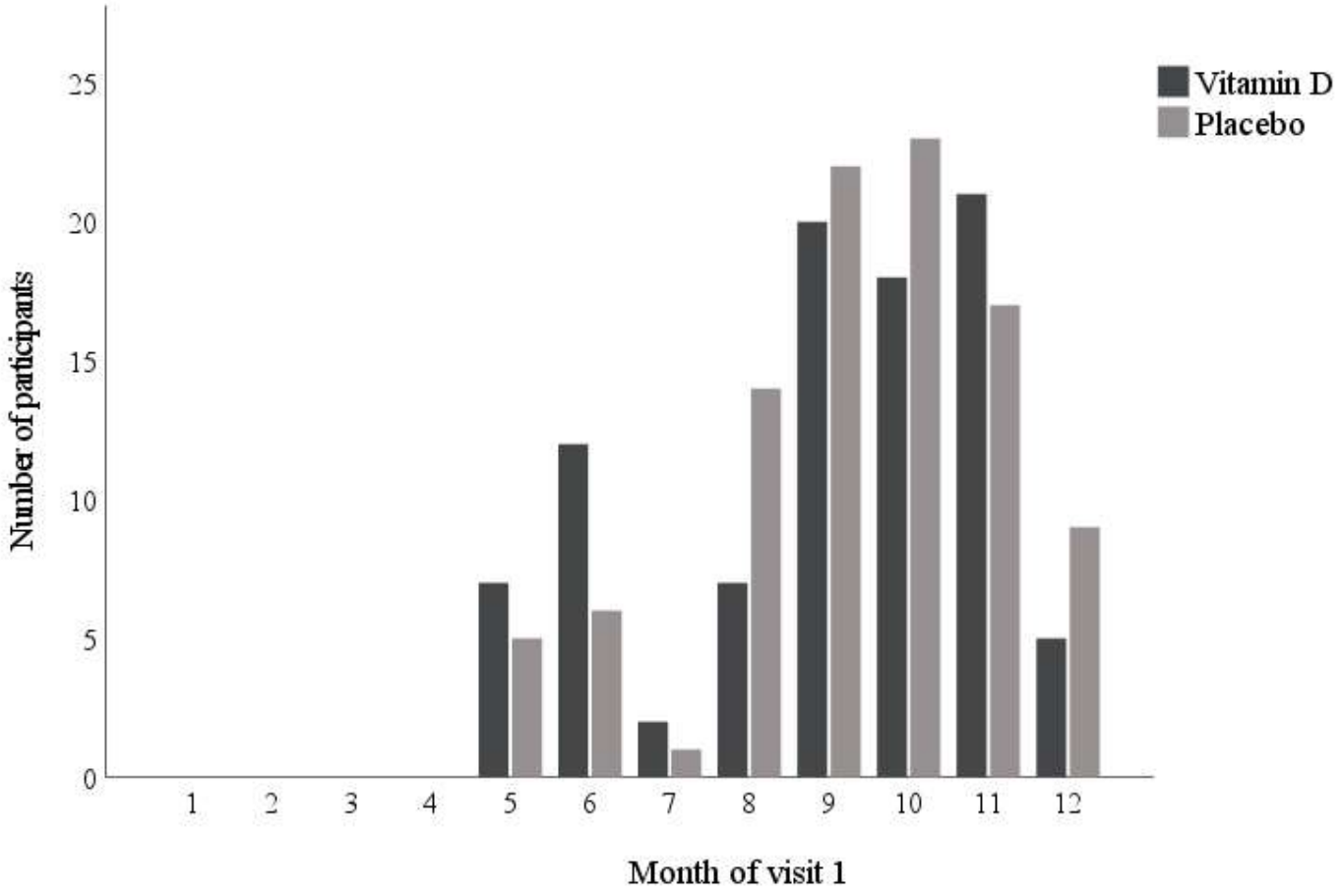
- ☐ Never or less than one night per month
- ☐ Less than one night per week
- ☐ 1-2 nights per week
- ☐ 3-5 nights per week
- ☐ Daily or almost daily

16. How likely are you to doze off or fall asleep in the following situation?	**No chance**	**Slight chance**	**Moderate chance**	**High chance**
Sitting and reading	☐	☐	☐	☐
Watching TV	☐	☐	☐	☐
Sitting inactive in a public place (e.g. theatre or meeting)	☐	☐	☐	☐
Lying down to rest in the afternoon when circumstances permit	☐	☐	☐	☐
When you are sitting and talking to someone	☐	☐	☐	☐
When you are sitting quietly after lunch without alcohol	☐	☐	☐	☐
When you are in a car, while stopped for a few minutes in traffic	☐	☐	☐	☐

17. Do you use any kind of sleeping medicine?

- ☐ Never
- ☐ Less than one night per month
- ☐ Less than one night per week
- ☐ 1-2 nights per week
- ☐ 3-5 nights per week
- ☐ Daily or almost daily

18. If you are using a sleep medicine, which one?



Supplemental Figure 2. Month of visit 1 (baseline) in the vitamin D group (*n* 92) (1=January, 2=February, etc.) and the placebo group (*n* 97) (1=January, 2=February, etc.) in participants with both baseline and end of study values for at least one sleep outcome (included between April 2016 and December 2016).

Paper III

Larsen AU, Grimnes G, Jorde R.

The effect of high-dose vitamin D_3 supplementation on bone mineral density in subjects with prediabetes.

Osteoporos Int 2017. 29(1):171-180.

Osteoporos Int
DOI 10.1007/s00198-017-4222-x

ORIGINAL ARTICLE

The effect of high-dose vitamin D_3 supplementation on bone mineral density in subjects with prediabetes

A. U. Larsen[1] · G. Grimnes[1,2] · R. Jorde[1,2]

Received: 27 April 2017 / Accepted: 6 September 2017

Abstract
Summary The rationale of this study was to determine the effect of high-dose vitamin D_3 supplementation on bone mineral density (BMD). Prediabetic males given vitamin D had significantly less reduction in BMD at the femoral neck compared to the controls. The clinical implications of our findings require further investigation.
Introduction Type 2 diabetes mellitus is associated with increased fracture risk, and recent studies show crosstalk between bone and glucose metabolism. Few studies have investigated the effect of vitamin D supplementation on the bone without additional calcium. In the present study, we aimed to determine whether a high dose of vitamin D_3 could improve bone mass density (BMD) in prediabetic subjects.
Methods The current study was conducted as a secondary research on a previously performed trial, in which 511 subjects with prediabetes were randomized to vitamin D_3 (20,000 IU per week) versus placebo for 5 years. BMD was measured using dual-energy X-ray absorptiometry (DEXA).
Results Two hundred and fifty-six subjects were randomized to vitamin D and 255 to placebo. Mean baseline serum 25-hydroxyvitamin D (25(OH)D) level was 60 nmol/L. Two hundred and two and 214 in the vitamin D and placebo groups, respectively, completed BMD measurements, whereas one in each group was excluded due to use of bisphosphonates. Males given vitamin D had significantly less reduction in BMD at the femoral neck measurement site compared to the controls (0.000 versus − 0.010 g/cm^2, $p = 0.008$). No significant differences between intervention groups were seen at the total hip measurement site, regarding both males and females.
Conclusions Vitamin D_3 supplementation alone may be beneficial in males with prediabetes, but confirmatory studies are needed.

Keywords Bone mineral density · Prediabetes · Randomized controlled trial · Vitamin D

Introduction

Diabetes mellitus is one of the world's most common chronic diseases, and overall prevalence among adults is estimated to increase in years to come [1, 2]. Blood glucose is, however, continuous, and type 2 diabetes mellitus (T2DM) develops through a prediabetic stage, defined by impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) [3, 4]. Such modest disturbances of glucose metabolism may increase the risk of complications traditionally attributed to T2DM, such as retinopathy, nephropathy, myocardial infarctions, and stroke, and both macro and microvascular damage appear to precede the onset of overt disease [5, 6].

Recently, it has been argued that the effects of chronically elevated glucose levels on the bone should be added to the more well-known complications of inadequately regulated glucose metabolism [7]. This is in line with the growing evidence of increased fracture risk in patients with T2DM,

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00198-017-4222-x) contains supplementary material, which is available to authorized users.

✉ A. U. Larsen
anette.uhlving@post.uit.no

[1] Tromsø Endocrine Research Group, Department of Clinical Medicine, UiT The Arctic University of Norway, 9037 Tromsø, Norway

[2] Division of Internal Medicine, University Hospital of North Norway, 9038 Tromsø, Norway

Published online: 18 September 2017

although these individuals are reported to have higher bone mineral density (BMD) than non-diabetic subjects [8–10]. It has been hypothesized that the accumulation of advanced glycation end products, impaired bone healing, and altered body composition, as well as an increased production of non-enzymatic cross-links within collagen fibers, have a negative impact on bone matrix properties [7]. Despite these findings, a recent meta-analysis exploring correlations of abnormal glucose metabolism reported no significant correlations neither with BMD nor with bone metabolism [11]. However, the increased propensity to fractures in patients with abnormal glucose metabolism may be caused by less apparent qualitative changes [12]. The notion of the bone being a true endocrine organ and an important regulator of whole-body glucose metabolism [13, 14] further complicates the relationship. In any case, improved bone health would be considered beneficial.

Vitamin D deficiency has been linked to both high blood glucose levels, insulin resistance, and greater risk of developing T2DM, although so far, the results of large RCTs do not support a causal relationship [15]. The role of vitamin D in maintenance of a healthy, mineralized skeleton through regulation of calcium and phosphate homeostasis is, however, well known. Moreover, vitamin D may contribute to improved bone health independent of its role in calcium homeostasis.

The active metabolite, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), has been suggested to exert local autocrine and paracrine regulation of bone turnover, in which $1,25(OH)_2D$ can stimulate both bone formation and resorption [16, 17]. Locally produced $1,25(OH)_2D$ is important for an optimized communication and coupling mechanism between osteoblasts and osteoclasts [18], as well as in osteoblast differentiation of human bone marrow cells [19, 20]. Moreover, $1,25(OH)_2D$ seems to affect secretion of osteoprotegerin from mature osteoblasts [21], and both the vitamin D receptor and the enzyme necessary for activation of 25(OH)D to $1,25(OH)_2D$, CYP27B1 (1-alphahydroxylase), are present in bone cells [16]. However, vitamin D may directly inhibit mineralization of the bone through increased local pyrophosphate concentrations [22], and the vitamin D-induced secretion of osteoprotegerin from osteoblasts has, together with RANKL, been suggested to stimulate osteoclastogenesis, thereby increasing bone resorption [23]. The latter also applies in states of vitamin D deficiency where secondary hyperparathyroidism arises, followed by a stimulated production of RANKL and osteoclastogenesis. Thus, vitamin D may exert biphasic effects, although consensus regarding this matter is yet to be reached.

In the present study, we hypothesized that supplementation with vitamin D could increase BMD in subjects with prediabetes, and thereby exert a preventive effect on fracture risk in this potentially exposed group.

Methods

Study design

The design of the study, where the main intention was to evaluate vitamin D for the prevention of T2DM, has been described in detail before [15, 24]. In short, prediabetic subjects (IFG (fasting serum glucose 6.0–6.9 mmol/L) and/or IGT (fasting serum glucose < 7.0 mmol/L and 2-h value 7.8–11.0 mmol/L at oral glucose tolerance test (OGTT) with 75 g glucose)) were included. Subjects were of both sexes, aged 25–80 years old. Most of them were recruited after participation in the sixth survey of the Tromsø Study (2007–2008) where 4393 subjects with hemoglobin A_{1c} (HbA_{1c}) in the range 5.0–6.9% (39.9–51.9 mmol/mol) and not previously diagnosed with diabetes, were invited to an OGTT, which was completed in 3476 subjects. Among these, 713 had IFG and/or IGT and were invited by letter to participate in the present study. In addition, a few other subjects were invited based on follow-up OGTTs performed in participants in previous studies [25, 26]. Subjects with primary hyperparathyroidism, granulomatous disease, history of urolithiasis, cancer diagnosed in the past 5 years, unstable angina pectoris, myocardial infarction, or stroke in the past year were excluded. Pregnant or lactating women, or women of fertile age with no use of contraception, were not included.

At the first visit, a brief clinical examination was performed, and questionnaires were filled in. The latter included questions on medical history and use of dietary supplementations. Height and weight were measured wearing light clothing. Fasting blood samples had been collected at the OGTT, and supplementary non-fasting blood samples were drawn at this first visit in the study. In all subjects, BMD was measured at baseline and at their last visit in the study with dual-energy X-ray absorptiometry (DEXA) (GE Lunar Prodigy, Lunar Corporation, Madison, WI, USA) at the femoral neck and total hip measurement site. The scanner was calibrated daily against the standard calibration block supplied by the manufacturer (aluminum spine phantom), and these measurements showed no drift throughout the study. The subjects were then randomized (non-stratified) in a 1:1 ratio to one capsule vitamin D_3 (cholecalciferol 20,000 IU per week (Dekristol; Mibe, Jena, Germany)) or an identical-looking placebo capsule containing arachis oil (Hasco-Lek, Wroclaw, Poland). New medication was supplied every sixth month, and unused capsules were returned and counted. The subjects were instructed not to take vitamin D supplements (including cod liver oil) exceeding 400 IU per day during the study.

For the next 5 years, the subjects met annually for new OGTTs, supplemental serum sampling, and height, weight, and blood pressure measurements. As part of a safety monitoring, serum calcium and creatinine were measured every sixth month, in between annual visits. At the annual visits,

all subjects were asked to fill in the same questionnaires as at the baseline visit. Adverse events were specifically asked for at all visits.

If at the annual OGTT the fasting blood glucose was > 6.9 mmol/L and/or the 2-h value > 11.0 mmol/L, the subject was considered to have T2DM, thus ending their participation in the study. These subjects were thereafter retested (if necessary) and followed by their general practitioner. HbA_{1c} was implemented in the present study as a diagnostic criterion from November 2012 [15], and thereafter, subjects were retested with a new HbA_{1c} measurement if HbA_{1c} alone was ≥ 6.5%. If still ≥ 6.5% after retest, subjects were diagnosed with T2DM, thereby ending their participation. Also, if diagnosed elsewhere with T2DM in between visits, participation in the study was ended.

Subjects with persistent measurements of serum calcium > 2.55 mmol/L were excluded, as well as subjects who developed renal stones, or symptoms compatible with renal stones. In the initial protocol, subjects who during the study were diagnosed with cancer, coronary infarction, unstable angina pectoris, or stroke were to be excluded from the study. From October 2011, this was changed to exclusion of subjects who during the study developed serious disease making it difficult or impossible to attend scheduled visits.

Biochemical analyses including serum 25(OH)D were analyzed using the gold standard LC-MSMS method, as previously described [15].

Statistical analyses

Normal distribution was evaluated by visual inspection of histograms, and by kurtosis and skewness. Log transformation was performed where appropriate. Comparisons of intervention groups at baseline were performed with Student's *t* test for continuous variables, Pearson's chi-square test for categorical variables, and Mann-Whitney *U* test for variables with a non-normal distribution. For BMD, the mean value of left and right measures was used for statistical analyses (when both values could be obtained). If only one side could be measured, this value was chosen to represent the mean value. Initially, measurements were to be classified as normal if corresponding to a T-score ≥ − 1.0, and if corresponding to a T-score between − 1.0 and − 2.5 or ≤ − 2.5 as osteopenic or osteoporotic, respectively [27]. However, since no male subjects and only very few female subjects presented with osteoporotic T-scores, all subjects with T-scores < − 1.0 were classified as osteopenic. Participants reporting use of bisphosphonates during the study were excluded from all statistical analyses. Predictors of baseline BMD were evaluated with multiple linear regression, applying forced entry on all predictor variables. Regarding change in BMD (delta values calculated as BMD at the last visit in the study minus BMD at baseline), comparison of the vitamin D and the placebo group was done using Student's *t* test. If significant, change in BMD was further tested with a linear regression model adjusting for baseline values [28], observation time and variables significantly predicting BMD at baseline (Table 2). All subgroups were analyzed likewise. The incidence of fractures during the study in the vitamin D and the placebo group was tested with a binary logistic regression analysis, adjusted for age and BMI.

A power calculation was made for the main endpoint (development of T2DM) [15], but a separate power calculation regarding BMD was not made. All tests were done two-sided, and $p < 0.05$ was considered statistically significant.

Statistical analyses were performed per protocol, using SPSS software version 24 (IBM Corp, Chicago, IL).

Ethics

Written informed consent for participation in the study was provided by all subjects who accepted the invitation. The study was approved by the Regional Committee for Medical Research Ethics (REK NORD 81/2007) and by the Norwegian Medicines Agency (2007-002167-27). The trial is registered at ClinicalTrials.gov (NCT00685594).

Results

A total of 511 subjects were included in the main study on prevention of T2DM. Ninety-five subjects were excluded due to missing baseline and/or final BMD measurements, and an additional two subjects were excluded due to use of bisphosphonates, thus leaving 414 subjects (201 given vitamin D and 213 given placebo) for the BMD analyses in the present study. Among these, 111 in the vitamin D group and 109 in the placebo group completed the 5-year intervention period. The flow of the study is shown in Fig. 1. Median observation time was 59 months in both of the male intervention groups ($p = 0.738$), while a non-significant difference in observation time was found between the female intervention groups with a median of 59 months in the vitamin D group versus 48 months in the placebo group ($p = 0.177$).

Baseline characteristics of the study participants are shown in Table 1, and no significant differences between the vitamin D and the placebo group were observed. The baseline serum 25(OH)D levels were 59.7 ± 22.0 nmol/L in the vitamin D group and 61.5 ± 20.4 nmol/L in the placebo group. During the 5-year intervention, mean serum 25(OH)D levels in the vitamin D group increased to 114.7 ± 27.4 nmol/L, whereas only minor changes were observed in mean serum 25(OH)D levels in the placebo group, as shown for males in Figs. 2 and 3. After 1 year, median serum PTH fell from 5.3 ± 2.1 to 5.0 ± 1.8 pmol/L in the vitamin D group, in contrast to an increase from 5.1 ± 2.1 to 5.2 ± 2.2 pmol/L in the placebo group ($p = 0.005$). A similar difference persisted throughout

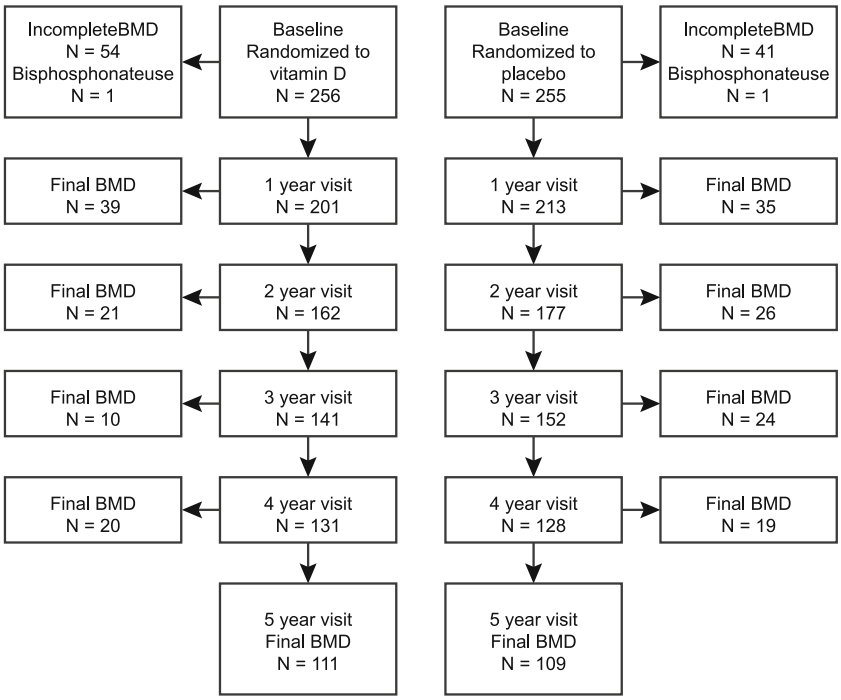


Fig. 1 Flowchart of the study

the study, both in men and women. The compliance rate was between 95 and 99% at all visits in both groups.

The baseline characteristics of the 97 subjects excluded due to missing BMD measurements can be found in Supplemental Table 1. Among these, there were no significant differences between the 55 subjects given vitamin D and the 42 subjects given placebo, nor were there any significant differences between the included (414 subjects) and the excluded (97 subjects) at baseline.

Among the entire study population, a total of 3885 adverse events were recorded during the 5-year intervention period, with no significant differences between intervention groups. Adverse events and side effects, including serious and/or calcium-specific events, have been described in detail before, and no serious side effects related to the intervention were recorded [15]. In the present study, we looked specifically at incident fractures. A total of 22 fractures were recorded among the subjects with valid BMD measurements, of which nine were in men. Of these nine, three fractures were recorded in the vitamin D group, against six in the placebo group. There was no significant difference in the number of fractures between the vitamin D group and the placebo group (adjusting for age, weight, and height); neither in general ($p = 0.868$) nor in stratified analyses (males, $p = 0.384$ versus females, $p = 0.249$).

BMD measurements

There was a non-significant trend ($p = 0.06$) for interaction between gender and treatment versus BMD at the femoral neck site, and thus, we chose to compare intervention groups regarding change in mean BMD separately for men and women. Body mass index (BMI) and tobacco use were found to significantly predict baseline BMD at the femoral neck and total hip measurement site in both sexes. Age significantly predicted baseline BMD at the femoral neck and total hip in females, whereas predicting baseline BMD only at the femoral neck measurement site in males. Additionally, baseline BMD in males was significantly predicted by serum calcium, PTH, and creatinine at both measurement sites (Table 2). There were no statistically significant differences in baseline BMD in the vitamin D and placebo group neither at the femoral neck, nor at the total hip (Table 3).

In males given vitamin D, there was no reduction in BMD at the femoral neck from baseline to the last visit in the study, values being 0.974 g/cm^2 at both visits respectively (Table 3). With adjustment for baseline BMD, observation time, and statistically significant predictors of baseline BMD (Table 2), this change differed significantly ($p = 0.008$) from that in the placebo group, of which corresponding values were 0.984 g/cm^2 at baseline and 0.973 g/cm^2 at the final visit (Table 3). At the total hip measurement site, a marginal difference was found between males given vitamin D versus placebo (an increase from 1.063 g/cm^2 at baseline to 1.065 g/cm^2 at final measurement in the vitamin D group versus a reduction from 1.078 to 1.075 g/cm^2 in the placebo group). However, this difference did not reach statistical significance ($p = 0.130$).

Regarding females, no significant differences were found between the two groups at either measurement site (Table 3).

Table 1 Baseline characteristics of the 414 study subjects

Variables	Males			Females		
	Vitamin D group (n = 125)	Placebo group (n = 131)	p value	Vitamin D group (n = 76)	Placebo group (n = 82)	p value
Age (years)	61.1 ± 7.6	61.0 ± 8.8	0.980	62.8 ± 8.3	63.1 ± 9.2	0.841
BMI (kg/m^2)	30.0 ± 3.8	30.1 ± 4.4	0.813	30.1 ± 4.3	29.4 ± 4.7	0.311
Tobacco use (%)	24.8	19.1	0.269	18.2	15.9	0.668
Femoral neck BMD (g/cm^2)	0.974 ± 0.126	0.984 ± 0.136	0.561	0.918 ± 0.117	0.887 ± 0.137	0.137
Total hip BMD (g/cm^2)	1.063 ± 0.137	1.078 ± 0.133	0.393	1.003 ± 0.129	0.961 ± 0.140	0.055
Osteopenia femoral neck (%)	4.8	6.1	0.646	5.3	8.5	0.419
Osteopenia total hip (%)	12.0	14.5	0.555	17.1	12.2	0.382
Serum 25(OH)D (nmol/L)	58.5 ± 23.0	59.0 ± 18.3	0.860	61.7 ± 20.3	65.7 ± 23.0	0.258
Vitamin D supplement use[a] (%)	30.4	38.9	0.152	36.8	28.0	0.238
Serum calcium, mmol/L	2.31 ± 0.07	2.30 ± 0.08	0.239	2.31 ± 0.07	2.32 ± 0.10	0.573
Calcium supplement use (%)	3.2	6.1	0.271	15.8	24.4	0.179
Serum PTH[b] (pmol/L)	5.3 (2.2)	5.2 (2.3)	0.443	5.7 (2.2)	5.2 (2.9)	0.514
Serum creatinine (μmol/L)	74.3 ± 12.9	75.4 ± 12.3	0.504	61.0 ± 9.7	61.1 ± 10.8	0.947
HbA_{1c} (%)	6.0 ± 0.3	5.9 ± 0.3	0.097	6.0 ± 0.3	6.0 ± 0.4	0.374
HbA_{1c} (mmol/mol)	42.0 ± 3.0	41.0 ± 3.0	–	42.0 ± 3.0	42.0 ± 4.0	–

Numbers represent mean ± SD, unless otherwise specified. Osteopenia T-score < −1.0

BMI body mass index, *BMD* bone mass density, *25(OH)D* 25-hydroxyvitamin D, *PTH* parathyroid hormone, *HbA_{1c}* hemoglobin A_{1c}

[a] Including cod liver oil

[b] Non-normal distribution, numbers represent median (IQR)

Subgroup analyses

A subgroup analysis was performed to investigate whether a more pronounced effect of vitamin D on BMD could be detected if including only subjects with 25(OH)D levels below 50 nmol/L. Thus, 68 subjects (47 males) in the vitamin D group and 63 (40 males) in the placebo group had serum 25(OH)D levels < 50 nmol/L at baseline (Supplemental Table 2). There were no significant differences between the intervention groups at baseline, and although the same trend was observed, with a marginal increase in BMD during the study among the males given vitamin D (data not shown), the difference versus the placebo group did not reach statistical significance (p = 0.072).

Due to the unique opportunity of investigating the effect of vitamin D supplementation on BMD without any supplemental dietary calcium, an additional subgroup analysis was performed, excluding users of calcium supplements at baseline and during the study. This analysis rendered 177 subjects (116 males) in the vitamin D group and 177 subjects (118 males) in the placebo group. The two groups were similar at baseline (Supplemental Table 3), and statistical regression analyses rendered the same results as in the main analyses regarding predictors of baseline mean BMD. Also, a statistically significant interaction term persisted between gender and intervention (p = 0.048), and stratified linear regression analyses produced the same results as when calcium users were included, with a statistically significant change in BMD at the femoral neck in men (p = 0.019), but not at other measurement sites and with no significant effects in women (data not shown).

Moreover, to investigate whether the difference between the vitamin D and the placebo group differed depending on their prediabetes-diagnosis, the cohort was split into three groups including those with (1) elevated fasting blood glucose only (6.0–6.9 mmol/L), (2) elevated 2-h values only (7.8–11.0 mmol/L), and (3) elevated measurements of both fasting and 2-h values of blood glucose. The sub-cohorts were then analyzed separately (applying the same statistical methods as in the main analyses) comparing delta BMD at the femoral neck and total hip in the vitamin D versus the placebo group. However, as the results were non-significant, these data are not shown.

Finally, subgroup analyses including only subjects with T-scores < − 1.0 were also carried out; however, few subjects were eligible for such analyses (Table 1), and no significant effects were detected at either measurement site (data not shown).

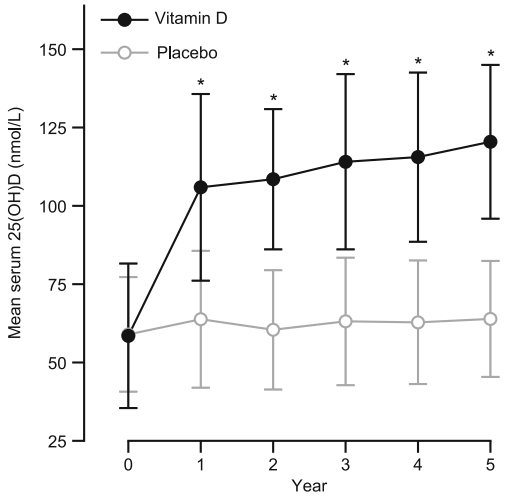


Fig. 2 Mean serum 25(OH)D levels during the study in the 125 males in the vitamin D group and the 131 males in the placebo group. Error bars represent 1 SD. Asterisks indicate $p < 0.001$ versus the control group with Student's *t* test

Discussion

In the present study, we hypothesized that supplementation with vitamin D could increase BMD in subjects with prediabetes, and we found a small, but significant positive effect of vitamin D supplementation on femoral neck BMD in males. To our knowledge, this has not been shown before. At the total hip measurement site, a positive, but non-significant effect was found. In the females, the vitamin D and the placebo group did not differ significantly.

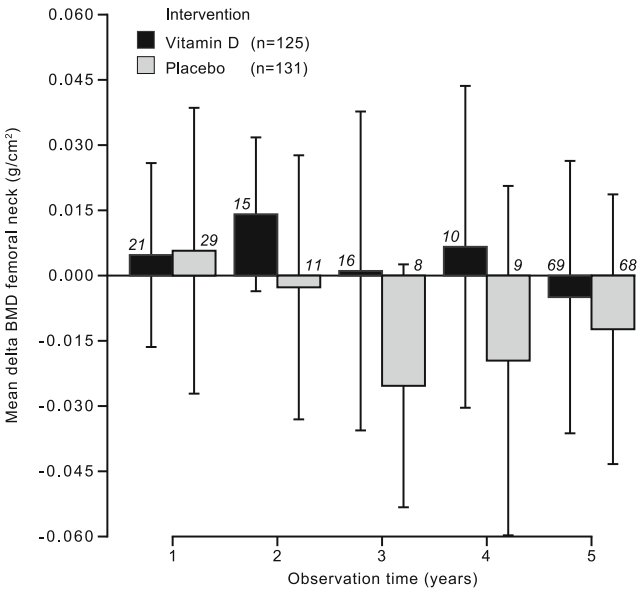


Fig. 3 Mean delta BMD (calculated as BMD at the last visit in the study minus BMD at baseline) at the femoral neck measurement site in the male intervention groups stratified by length of intervention. The number on top of the bars represents the number of participants in each subgroup. Error bars represent 1 SD

To our knowledge, there has only been a few other RCTs where the effect on BMD of vitamin D given alone has been studied. Thus, in the review and meta-analysis by Reid et al. in 2014 [29], 23 studies were identified where the interventions differed only in vitamin D content. However, vitamin D was given alone without calcium or other co-interventions in only seven studies, and among these, none but three included males. Of the studies including males, one included 50 subjects randomized to 300,000 IU vitamin D per year [30] and was excluded from the meta-analysis because of a 9-year age difference between the intervention groups; another included 173 subjects randomized to 400 IU, 800 IU, or placebo over 12 months where a non-significant positive effect at the lumbar spine and a significant negative effect at total BMD was found, however, not including measurements at the femoral neck [31]; and the third study was excluded due to not available nor obtainable quantitative data in the original publication [32]. As far as we are concerned, there has not been any studies with vitamin D alone that has included males published since 2014, and therefore it is fair to say that this has not been properly examined before.

In the present study, a positive effect of vitamin D supplementation was found only at the femoral neck measurement site. The femoral neck contains more cortical bone than what is included in the total hip measurement. The cortical bone is metabolically less active than the trabecular bone [33], and previous studies have shown that the cortical bone is also less responsive to treatment than the trabecular bone [34]. However, in the case of vitamin D deficiency, the secondary hyperparathyroidism causes bone loss mainly at cortical sites [35], and suppression of PTH, as was seen in our vitamin D group, could be the explanation for the BMD increase in the femoral neck. This was also found in the study by Ooms et al. [36] where vitamin D_3 400 IU/day versus placebo led to an increase of femoral neck BMD of 2% after 2 years, while there was no change at the trochanter. Moreover, these observations fit with the conclusion in the review by Reid et al. [29], in which a small, but significant effect was found at the femoral neck, but not at other measurement sites.

Yet, some limitations of our study ought to be considered. First, change in BMD was not the primary endpoint; thus, the study design may not have been appropriate. The inclusion criteria (IFG/IGT) favored subjects with high BMI, which is traditionally observed to have higher BMD due to mechanical loading and estrogen production via adipocyte aromatization [37]. Moreover, only a small number of subjects presented T-values corresponding with osteopenia, and accordingly, major improvements in BMD may therefore not have been likely. The influence of adipose tissue on bone metabolism is, however, not yet settled as recent studies indicate an inverse association between increased adiposity and low total BMD and total bone mineral content [38]. Additionally, studies have shown that T2DM patients are at higher risk of fracture when

Table 2 Predictors of baseline BMD in male and female subjects

Variables	Males				Females			
	Femoral neck (g/cm^2)	*p* value	Total hip (g/cm^2)	*p* value	Femoral neck (g/cm^2)	*p* value	Total hip (g/cm^2)	*p* value
Age (years)	− 0.180	0.003*	− 0.112	0.068	− 0.411	< 0.001*	− 0.328	< 0.001*
BMI (kg/m^2)	0.313	tbcolw30pt< 0.001*	0.334	tbcolw30pt< 0.001*	0.258	< 0.001*	0.336	< 0.001*
Tobacco use[e] (%)	− 0.128	0.029*	− 0.148	0.013*	− 0.151	0.035*	− 0.143	0.047*
tbcolw110ptVitamin D supplement use[a,b] (%)	0.025	0.668	− 0.021	0.715	− 0.005	0.943	− 0.047	0.519
Calcium supplement use[e] (%)	− 0.075	0.200	− 0.089	0.133	− 0.109	0.139	− 0.132	0.074
Serum 25(OH)D (nmol/L)	− 0.006	0.922	− 0.007	0.906	− 0.049	0.534	− 0.047	0.551
Serum calcium (mmol/L)	− 0.176	0.003*	− 0.128	0.032*	− 0.014	0.846	− 0.040	0.577
Serum PTH (pmol/L)	− 0.177	0.004*	− 0.127	0.041*	− 0.161	0.051	− 0.126	0.126
Serum creatinine (μmol/L)	0.171	0.005*	0.131	0.033*	0.119	0.121	0.059	0.445
HbA_{1c} (%)	0.006	0.921	0.059	0.313	0.101	0.165	0.097	0.185
R^2	0.238	< 0.001	0.216	< 0.001	0.330	< 0.001	0.323	< 0.001

Numbers represent standardized beta-coefficients and associated *p* values

[a] Including cod liver oil

[b] Coding: 0 = No, 1 = Yes

*Variable included in the linear regression model

they have incorrectly treated glucose levels [7]. Thus, an effect of vitamin D on fracture risk may have been shadowed in the present trial, as it was originally designed to remove all subjects developing T2DM.

Second, low serum 25(OH)D level was not an inclusion criteria at baseline, resulting in a wide range of serum 25(OH)D levels among the study participants. Baseline serum 25(OH)D levels were relatively high in the study population,

Table 3 Bone mass density measurements

		Vitamin D group	Placebo group	*p* value
Males (*n*)		125	131	
Femoral neck BMD (g/cm^2)	Baseline	0.974 ± 0.126	0.984 ± 0.136	0.561[a]
	Last visit	0.974 ± 0.124	0.973 ± 0.137	
	Delta	0.000 ± 0.029	− 0.010 ± 0.032	0.008[b,c]
Total hip BMD (g/cm^2)	Baseline	1.063 ± 0.137	1.078 ± 0.133	0.393[a]
	Last visit	1.065 ± 0.141	1.075 ± 0.14	
	Delta	0.002 ± 0.024	− 0.003 ± 0.03	0.149[a]
Females (*n*)		76	82	
Femoral neck BMD (g/cm^2)	Baseline	0.918 ± 0.117	0.887 ± 0.137	0.137[a]
	Last visit	0.900 ± 0.120	0.873 ± 0.143	
	Delta	− 0.017 ± 0.034	− 0.015 ± 0.029	0.592[a]
Total hip BMD (g/cm^2)	Baseline	1.003 ± 0.129	0.961 ± 0.140	0.055[a]
	Last visit	0.986 ± 0.130	0.943 ± 0.150	
	Delta	− 0.017 ± 0.034	− 0.018 ± 0.029	0.813[a]

Numbers represent mean ± SD

BMD bone mass density (g/cm^2), *Delta* $BMD_{Last\ visit} - BMD_{Baseline}$

[a] Student's *t* test

[b] Linear regression adjusting for baseline values, observation time and predictors of baseline BMD (Table 2)

[c] $R^2 = 0.082$

and thus, one might not expect major effects of further supplementation with vitamin D. Nevertheless, a small positive effect on BMD was observed in men. Moreover, subgroup analyses of data from subjects with baseline serum 25(OH)D levels below 50 nmol/L did not show significant effects; however, this might be explained by lack of statistical power, as the subgroup consisted of only a small number of subjects.

Third, length of intervention also varied among the study participants, with approximately 53% completing the 5-year trial. In short, median observation time was the same in the vitamin D group compared to the placebo group in males, while being longer in the female vitamin D group compared to placebo. However, BMD was found to increase in the male vitamin D group only, and when comparing median observation time between intervention groups, differences were non-significant for both men and women.

Fourth, the effect of vitamin D supplementation on BMD was not observed in both sexes. However, sexual dimorphism is not a new nor surprising finding when it comes to skeletal physiology and bone metabolism [39]. On average, men are taller, have larger amounts of muscle mass and lower body fat percentage, as well as having greater peak bone mineral content and peak trabecular bone volume [40–43]. The establishment of gender differences in the cortical and trabecular bone is found to be regulated by androgen and estrogen bioactivity, through a dual mode of action of testosterone on the cortical and trabecular bone via both the androgen receptor and estrogen receptor alpha [37]. If regulation of bone turnover in women operates through more complex mechanisms than in men, these mechanisms might override potential effects of vitamin D supplementation on the bone in women. However, information regarding history of use and/or current use of hormonal drugs was not available in the present study, and adjustments for these factors was therefore not made.

Finally, the proportion of variance of BMD explained (R^2) by the models in our analyses was rather small, and the clinical implications of our findings may be of modest importance. A small increase in BMD does not necessarily mean successful prevention of falls and/or fractures, as the reduction in bone strength is not only determined by BMD, but also by bone dimensions, microstructure, and material properties [37]. DEXA is a projectional (two-dimensional) technique, and thus, cannot truly differentiate between the cortical and trabecular bone. Therefore, such measures of bone health cannot assess the less apparent qualitative changes that may be present due to impaired glucose metabolism. Unfortunately, measurements with techniques allowing a three-dimensional assessment of bone structure and microarchitecture, such as peripheral quantitative computed tomography (pQCT) scanning, were not available in the present study.

However, the study has some strengths, as it is the largest, longest-running, published RCT with vitamin D as the only intervention, and both dosage and length of intervention ought to have been sufficient in order to detect an actual effect on BMD.

In conclusion, we have found a positive effect of vitamin D on BMD in males, but confirmatory studies are needed, preferably with change in BMD as the primary endpoint, and levels of 25(OH)D below 50 nmol/L as inclusion criterion. Additionally, evaluating bone properties with other techniques, such as high-resolution pQCT, may provide valuable insights.

Acknowledgements The authors gratefully acknowledge the superb assistance from the staff at the Clinical Research Unit (in particular Aslaug Jakobsen) and the Department of Medical Biochemistry at the University Hospital of North Norway.

Funding The study was supported by grants from the Novo Nordisk Foundation (Grant R195-A16126), the North Norway Regional Health Authorities (Grant 6856/SFP1029-12), UiT The Arctic University of Norway, the Norwegian Diabetes Association, and the Research Council of Norway (Grant 184766).

Compliance with ethical standards

Conflicts of interest None.

References

1. Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE (2014) Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res Clin Pract 103:137–149. https://doi.org/10.1016/j.diabres.2013.11.002
2. Chen L, Magliano DJ, Zimmet PZ (2011) The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives. Nat Rev Endocrinol 8:228–236. https://doi.org/10.1038/nrendo.2011.183
3. Knowler WC, Barett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM (2002) Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 346:393–403. https://doi.org/10.1056/NEJMoa012512
4. Morris DH, Khunti K, Achana F, Srinivasan B, Gray LJ, Davies MJ, Webb D (2013) Progression rates from HbA1c 6.0–6.4% and other prediabetes definitions to type 2 diabetes: a meta-analysis. Diabetologia 56:1489–1493. https://doi.org/10.1007/s00125-013-2902-4
5. Singleton JR, Smith AG, Russell JW, Feldman EL (2003) Microvascular complications of impaired glucose tolerance. Diabetes 52:2867–2873. https://doi.org/10.2337/diabetes.52.12.2867
6. Plantinga LC, Crews DC, Coresh J, Miller ER, Saran R, Yee J, Hedgeman E, Pavkov M, Eberhardt MS, Williams DE (2010) Prevalence of chronic kidney disease in US adults with undiagnosed diabetes or prediabetes. Clin J Am Soc Nephrol 5:673–682. https://doi.org/10.2215/CJN.07891109
7. Oei L, Zillikens MC, Dehghan A, Buitendijk GH, Castano-Betancourt MC, Estrada K, Stolk L, Oei EH, van Meurs JB, Janssen JA, Hofman A, van Leeuwen JP, Witteman JC, Pols HA, Uitterlinden AG, Klaver CC, Franco OH, Rivadeneira F (2013) High bone mineral density and fracture risk in type 2 diabetes as skeletal complications of inadequate glucose control: the Rotterdam study. Diabetes Care 36:1619–1628. https://doi.org/10.2337/dc12-1188

8. Janghorbani M, Van Dam RM, Willett WC, Hu FB (2007) Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture. Am J Epidemiol 166:495–505. https://doi.org/10.1093/aje/kwm106
9. Schwartz AV, Vittinghoff E, Bauer DC, Hillier TA, Strotmeyer ES, Ensrud KE, Donaldson MG, Cauley JA, Harris TB, Koster A, Womack CR, Palermo L, Black DM (2011) Association of BMD and FRAX score with risk of fracture in older adults with type 2 diabetes. JAMA 305:2184–2192. https://doi.org/10.1001/jama.2011.715
10. Ma L, Oei L, Jiang L, Estrada K, Chen H, Wang Z, Yu Q, Zillikens MC, Gao X, Rivadeneira F (2012) Association between bone mineral density and type 2 diabetes mellitus: a meta-analysis of observational studies. Eur J Epidemiol 27:319–332. https://doi.org/10.1007/s10654-012-9674-x
11. Qu Y, Kang MY, Dong RP, Zhao JW (2016) Correlations between abnormal glucose metabolism and bone mineral density or bone metabolism. Med Sci Monit 22:824–832
12. Merlotti D, Gennari L, Dotta F, Lauro D, Nuti R (2010) Mechanisms of impaired bone strength in type 1 and 2 diabetes. Nutr Metab Cardiovasc Dis 20:683–690. https://doi.org/10.1016/j.numecd.2010.07.008
13. Wei J, Ferron M, Clarke CJ, Hannun YA, Jiang H, Blaner WS, Karsenty G (2014) Bone-specific insulin resistance disrupts whole-body glucose homeostasis via decreased osteocalcin activation. J Clin Invest 124:1–13. https://doi.org/10.1172/jci72323
14. Oldknow KJ, MacRae VE, Farquharson C (2015) Endocrine role of bone: recent and emerging perspectives beyond osteocalcin. J Endocrinol 225:R1–R19. https://doi.org/10.1530/joe-14-0584
15. Jorde R, Sollid ST, Svartberg J, Schirmer H, Joakimsen RM, Njolstad I, Fuskevag OM, Figenschau Y, Hutchinson MY (2016) Vitamin D 20,000 IU per week for five years does not prevent progression from prediabetes to diabetes. J Clin Endocrinol Metab 101:1647–1655. https://doi.org/10.1210/jc.2015-4013
16. Anderson PH, Atkins GJ (2008) The skeleton as an intracrine organ for vitamin D metabolism. Mol Asp Med 29:397–406. https://doi.org/10.1016/j.mam.2008.05.003
17. Grimnes G, Joakimsen R, Figenschau Y, Torjesen PA, Almas B, Jorde R (2012) The effect of high-dose vitamin D on bone mineral density and bone turnover markers in postmenopausal women with low bone mass—a randomized controlled 1-year trial. Osteoporos Int 23:201–211. https://doi.org/10.1007/s00198-011-1752-5
18. Kogawa M, Findlay DM, Anderson PH, Ormsby R, Vincent C, Morris HA, Atkins GJ (2010) Osteoclastic metabolism of 25(OH)-vitamin D3: a potential mechanism for optimization of bone resorption. Endocrinology 151:4613–4625. https://doi.org/10.1210/en.2010-0334
19. Zhou S, LeBoff MS, Glowacki J (2010) Vitamin D metabolism and action in human bone marrow stromal cells. Endocrinology 151:14–22. https://doi.org/10.1210/en.2009-0969
20. Geng S, Zhou S, Glowacki J (2011) Effects of 25-hydroxyvitamin D(3) on proliferation and osteoblast differentiation of human marrow stromal cells require CYP27B1/1alpha-hydroxylase. J Bone Miner Res 26:1145–1153. https://doi.org/10.1002/jbmr.298
21. Baldock PA, Thomas GP, Hodge JM, Baker SU, Dressel U, O'Loughlin PD, Nicholson GC, Briffa KH, Eisman JA, Gardiner EM (2006) Vitamin D action and regulation of bone remodeling: suppression of osteoclastogenesis by the mature osteoblast. J Bone Miner Res 21:1618–1626. https://doi.org/10.1359/jbmr.060714
22. Lieben L, Masuyama R, Torrekens S, Van Looveren R, Schrooten J, Baatsen P, Lafage-Proust MH, Dresselaers T, Feng JQ, Bonewald LF, Meyer MB, Pike JW, Bouillon R, Carmeliet G (2012) Normocalcemia is maintained in mice under conditions of calcium malabsorption by vitamin D-induced inhibition of bone mineralization. J Clin Invest 122:1803–1815. https://doi.org/10.1172/jci45890
23. Haussler MR, Haussler CA, Whitfield GK, Hsieh JC, Thompson PD, Barthel TK, Bartik L, Egan JB, Wu Y, Kubicek JL, Lowmiller CL, Moffet EW, Forster RE, Jurutka PW (2010) The nuclear vitamin D receptor controls the expression of genes encoding factors which feed the "Fountain of Youth" to mediate healthful aging. J Steroid Biochem Mol Biol 121:88–97. https://doi.org/10.1016/j.jsbmb.2010.03.019
24. Sollid ST, Hutchinson MY, Fuskevag OM, Figenschau Y, Joakimsen RM, Schirmer H, Njolstad I, Svartberg J, Kamycheva E, Jorde R (2014) No effect of high-dose vitamin D supplementation on glycemic status or cardiovascular risk factors in subjects with prediabetes. Diabetes Care 37:2123–2131. https://doi.org/10.2337/dc14-0218
25. Sneve M, Figenschau Y, Jorde R (2008) Supplementation with cholecalciferol does not result in weight reduction in overweight and obese subjects. Eur J Endocrinol 159:675–684. https://doi.org/10.1530/eje-08-0339
26. Melsom T, Mathisen UD, Eilertsen BA, Ingebretsen OC, Jenssen T, Njolstad I, Solbu MD, Toft I, Eriksen BO (2012) Physical exercise, fasting glucose, and renal hyperfiltration in the general population: the Renal Iohexol Clearance Survey in Tromso 6 (RENIS-T6). Clin J Am Soc Nephrol 7:1801–1810. https://doi.org/10.2215/cjn.02980312
27. World Health Organization (1994) Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: report of a WHO study group. World Health Organ Tech Rep Ser 843:1–129
28. Vickers AJ, Altman DG (2001) Statistics notes: analysing controlled trials with baseline and follow up measurements. BMJ 323:1123–1124
29. Reid IR, Bolland MJ, Grey A (2014) Effects of vitamin D supplements on bone mineral density: a systematic review and meta-analysis. Lancet 383:146–155. https://doi.org/10.1016/s0140-6736(13)61647-5
30. Venkatachalam S, Gupta R, Speden D et al (2003) A randomised controlled trial of parenteral vitamin D in coeliac disease-bone density changes. Osteoporos Int 14(Suppl 4):S39–S40
31. Andersen R, Molgaard C, Skovgaard LT, Brot C, Cashman KD, Jakobsen J, Lamberg-Allardt C, Ovesen L (2008) Effect of vitamin D supplementation on bone and vitamin D status among Pakistani immigrants in Denmark: a randomised double-blinded placebo-controlled intervention study. Br J Nutr 100:197–207. https://doi.org/10.1017/s000711450789430x
32. Viljakainen HT, Vaisanen M, Kemi V, Rikkonen T, Kroger H, Laitinen EK, Rita H, Lamberg-Allardt C (2009) Wintertime vitamin D supplementation inhibits seasonal variation of calcitropic hormones and maintains bone turnover in healthy men. J Bone Miner Res 24:346–352. https://doi.org/10.1359/jbmr.081009
33. Reginster JY (1995) Treatment of bone in elderly subjects: calcium, vitamin D, fluor, bisphosphonates, calcitonin. Horm Res 43:83–88
34. Crilly RG, Cox L (2013) A comparison of bone density and bone morphology between patients presenting with hip fractures, spinal fractures or a combination of the two. BMC Musculoskelet Disord 14:68. https://doi.org/10.1186/1471-2474-14-68
35. Lips P (2001) Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocr Rev 22:477–501. https://doi.org/10.1210/edrv.22.4.0437
36. Ooms ME, Roos JC, Bezemer PD, van der Vijgh WJ, Bouter LM, Lips P (1995) Prevention of bone loss by vitamin D supplementation in elderly women: a randomized double-blind trial. J Clin Endocrinol Metab 80:1052–1058. https://doi.org/10.1210/jcem.80.4.7714065
37. Vanderschueren D, Laurent MR, Claessens F, Gielen E, Lagerquist MK, Vandenput L, Borjesson AE, Ohlsson C (2014) Sex steroid

actions in male bone. Endocr Rev 35:906–960. https://doi.org/10.1210/er.2014-1024
38. Cao JJ (2011) Effects of obesity on bone metabolism. J Orthop Surg Res 6:30. https://doi.org/10.1186/1749-799x-6-30
39. Riggs BL, Melton Iii LJ 3rd, Robb RA, Camp JJ, Atkinson EJ, Peterson JM, Rouleau PA, CH MC, Bouxsein ML, Khosla S (2004) Population-based study of age and sex differences in bone volumetric density, size, geometry, and structure at different skeletal sites. J Bone Miner Res 19:1945–1954. https://doi.org/10.1359/jbmr.040916
40. Khosla S, Amin S, Orwoll E (2008) Osteoporosis in men. Endocr Rev 29:441–464. https://doi.org/10.1210/er.2008-0002
41. Riggs BL, Khosla S, Melton LJ 3rd (2002) Sex steroids and the construction and conservation of the adult skeleton. Endocr Rev 23:279–302. https://doi.org/10.1210/edrv.23.3.0465
42. Lindle RS, Metter EJ, Lynch NA, Fleg JL, Fozard JL, Tobin J, Roy TA, Hurley BF (1997) Age and gender comparisons of muscle strength in 654 women and men aged 20-93 yr. J Appl Physiol 83:1581–1587
43. Visser M, Fuerst T, Lang T, Salamone L, Harris TB (1999) Validity of fan-beam dual-energy X-ray absorptiometry for measuring fat-free mass and leg muscle mass. Health, aging, and body composition study—dual-energy X-ray absorptiometry and body composition working group. J Appl Physiol 87:1513–1520

THE EFFECT OF HIGH-DOSE VITAMIN D_3 SUPPLEMENTATION ON BONE MINERAL DENSITY IN SUBJECTS WITH PREDIABETES. Osteoporos Int. A.U. Larsen, G. Grimnes, R. Jorde; Tromsø Endocrine Research Group, Department of Clinical Medicine, UiT – The Arctic University of Norway. anette.uhlving@uit.no

Supplemental Table 1. Baseline characteristics in subjects excluded due to lacking BMD measurements

	Males		Females	
	Vitamin D group	Placebo group	Vitamin D group	Placebo group
N	36	22	19	20
Age, years	65.4 ± 8.0	61.0 ± 11.0	61.5 ± 9.8	63.8 ± 9.8
BMI, kg/m^2	30.0 ± 3.9	30.4 ± 3.1	30.6 ± 5.2	28.8 ± 5.2
Tobacco use, %	27.8	22.7	21.1	20.0
Serum 25(OH)D, nmol/L	57.9 ± 22.4	52.4 ± 15.5	65.8 ± 19.0	65.6 ± 31.4
Vitamin D supplement use[a], %	38.9	45.5	36.8	40.0
Serum calcium, mmol/L	2.29 ± 0.09	2.32 ± 0.07	2.33 ± 0.08	2.33 ± 0.07
Calcium supplement use, %	11.1	9.1	10.5	20.0
Bisphosphonate use, %	5.6	0.0	0.0	10.0
Serum PTH[b], ρmol/L	5.8 (3.7)	5.4 (1.9)	5.9 (2.4)	5.7 (2.5)
Serum creatinine, µmol/L	77.2 ± 12.3	76.9 ± 14.7	59.7 ± 9.9	57.7 ± 9.0
HbA_{1c}, %	6.0 ± 0.3	6.0 ± 0.3	5.9 ± 0.3	6.1 ± 0.3
HbA_{1c} (mmol/mol)	42.0 ± 3.0	42.0 ± 3.0	41.0 ± 3.0	43.0 ± 3.0

a) Including cod liver oil

b) Non-normal distribution, numbers represent median (IQR)

Numbers represent mean ± SD, unless otherwise specified.

BMI: body mass index; BMD; bone mass density; 25(OH)D: 25-hydroxyvitamin D; PTH: parathyroid hormone; HbA_{1c}: haemoglobin A_{1c}.

Supplemental Table 2. Baseline characteristics of subjects with serum 25(OH)D level < 50 nmol/L

	Males		Females	
	Vitamin D group	Placebo group	Vitamin D group	Placebo group
N	47	40	21	23
Age, years	59.8 ± 7.9	58.9 ± 9.5	61.7 ± 8.6	60.7 ± 10.2
BMI, kg/m^2	29.9 ± 4.4	30.9 ± 4.2	31.2 ± 4.6	29.7 ± 2.7
Tobacco use, %	23.4	20.0	28.6	21.7
Femoral neck BMD, g/cm^2	0.975 ± 0.137	0.995 ± 0.143	0.917 ± 0.106	0.905 ± 0.141
Total Hip BMD, g/cm^2	1.066 ± 0.158	1.090 ± 0.126	1.003 ± 0.138	0.985 ± 0.150
Osteopenia femoral neck, %	8.5	7.5	0.0	13.0
Osteopenia total hip, %	17.0	17.5	23.8	13.0
Serum 25(OH)D, nmol/L	39.7 ± 8.8	40.0 ± 7.6	39.3 ± 6.5	38.2 ± 6.1
Vitamin D supplement use[a], %	27.7	30.0	28.6	8.7
Serum calcium, mmol/L	2.32 ± 0.07	2.27 ± 0.07	2.29 ± 0.06	2.32 ± 0.10
Calcium supplement use, %	4.3	10.0	14.3	26.1
Serum PTH[b], ρmol/L	5.6 (2.0)	5.9 (2.4)	6.0 (2.0)	7.0 (3.1)
Serum creatinine, µmol/L	72.3 ± 12.4	74.5 ± 10.9	58.5 ± 9.7	62.0 ± 12.7
HbA_{1c}, %	6.1 ± 0.2	5.9 ± 0.3	6.0 ± 0.3	6.2 ± 0.5
HbA_{1c}, mmol/mol	43.0 ± 2.0	41.0 ± 3.0	42.0 ± 3.0	44.0 ± 5.0

a) Including cod liver oil

b) Non-normal distribution, numbers represent median (IQR)

Numbers represent mean ± SD, unless otherwise specified.

BMI: body mass index; BMD; bone mass density; 25(OH)D: 25-hydroxyvitamin D; PTH: parathyroid hormone; HbA_{1c}: haemoglobin A_{1c}. Osteopenia; T-score < -1.0.

Supplemental Table 3. Baseline characteristics of subjects not using calcium supplements

	Males		Females	
	Vitamin D group	Placebo group	Vitamin D group	Placebo group
N	121	123	64	62
Age, years	61.1 ± 7.6	61.0 ± 8.6	61.9 ± 8.1	62.4 ± 9.4
BMI, kg/m^2	29.9 ± 3.7	30.4 ± 4.3	30.3 ± 4.3	29.0 ± 4.5
Tobacco use, %	25.6	20.3	18.8	16.1
Femoral neck BMD, g/cm^2	0.974 ± 0.126	0.990 ± 0.133	0.932 ± 0.114	0.893 ± 0.134
Total Hip BMD, g/cm^2	1.063 ± 0.138	1.085 ± 0.131	1.020 ± 0.118	0.968 ± 0.136
Osteopenia femoral neck, %	5.0	6.5	6.3	9.7
Osteopenia total hip, %	12.4	15.4	18.8	12.9
Serum 25(OH)D, nmol/L	58.8 ± 22.5	59.5 ± 17.5	60.6 ± 19.1	66.0 ± 23.7
Vitamin D supplement use[a], %	30.6	38.2	32.8	24.2
Serum calcium, mmol/L	2.31 ± 0.08	2.30 ± 0.08	2.31 ± 0.07	2.33 ± 0.10
Serum PTH[b], ρmol/L	5.3 (2.0)	5.2 (2.2)	5.7 (2.0)	5.2 (2.2)
Serum creatinine, µmol/L	74.3 ± 13.1	75.9 ± 12.3	60.6 ± 8.9	59.2 ± 10.0
HbA_{1c}, %	6.0 ± 0.3	5.9 ± 0.3	5.9 ± 0.3	6.0 ± 0.4
HbA_{1c}, mmol/mol	42.0 ± 3.0	41.0 ± 3.0	41.0 ± 3.0	42.0 ± 4.0

a) Including cod liver oil

b) Non-normal distribution, numbers represent median (IQR)

Numbers represent mean ± SD, unless otherwise specified.

BMI: body mass index; BMD; bone mass density; 25(OH)D: 25-hydroxyvitamin D; PTH: parathyroid hormone; HbA_{1c}: haemoglobin A_{1c}. Osteopenia; T-score < -1.0.

Appendix 1.

General questionnaire Q1 in Tromsø7 2015-16.

The Tromsø Study
2015–2016

CONFIDENTIAL

The questionnaire will be optically read. Please, use blue or black inked pen only. Use block lettering. Refrain from the use of comma.

Date for filling in the questionnaire: ☐☐☐☐☐☐

HEALTH AND DISEASES

1.1 How do you in general consider your health to be?

Excellent	Good	Neither good nor bad	Bad	Very bad
☐	☐	☐	☐	☐

1.2 How is your health now compared to others of your age?

Excellent	Good	Neither good nor bad	Bad	Very bad
☐	☐	☐	☐	☐

1.3 Have you ever had, or do you have?
Tick once for each line.

	No	Yes, currently	Previously, not now	Age first time
High blood pressure	☐	☐	☐	☐☐
Heart attack	☐		☐	☐☐
Heart failure	☐	☐	☐	☐☐
Atrial fibrillation	☐	☐	☐	☐☐
Angina pectoris *(heart cramp)*	☐	☐	☐	☐☐
Cerebral stroke / brain haemorrhage	☐		☐	☐☐
Diabetes	☐	☐	☐	☐☐
Kidney disease, not including urinary tract infection *(UTI)*	☐	☐	☐	☐☐
Bronchitis / emphysema / COPD	☐	☐	☐	☐☐
Asthma	☐	☐	☐	☐☐
Cancer	☐	☐	☐	☐☐
Rheumatoid Arthritis	☐	☐	☐	☐☐
Arthrosis	☐	☐	☐	☐☐
Migraine	☐	☐	☐	☐☐
Psychological problems for which you have sought help	☐	☐	☐	☐☐

1.4 Do you have persistent or constantly recurring pain that has lasted for three months or more?

☐ No ☐ Yes

DENTAL HEALTH

2.1 How do you consider your own dental health to be?

	1	2	3	4	5	
Very bad	☐	☐	☐	☐	☐	Excellent

2.2 How satisfied or dissatisfied are you with your teeth or denture?

	1	2	3	4	5	
Very dissatisfied	☐	☐	☐	☐	☐	Very satisfied

USE OF HEALTH SERVICES

3.1 Have you during the past 12 months visited?

	Yes	No	Number of times
General practitioner *(GP)*	☐	☐	☐☐
Emergency room	☐	☐	☐☐
Psychiatrist / Psychologist	☐	☐	☐☐
Another medical specialist than a general practitioner *(GP)* or a psychologist or psychiatrist *(not at a hospital)*	☐	☐	☐☐
Dentist / dental services	☐	☐	☐☐
Pharmacy *(to buy / get advice about medicines / treatment)*	☐	☐	☐☐
Physiotherapist	☐	☐	☐☐
Chiropractor	☐	☐	☐☐
Acupuncturist	☐	☐	☐☐
CAM provider *(homeopath, reflexologist, spiritual healer etc.)*	☐	☐	☐☐
Traditional healer *(helper, "reader" etc.)*	☐	☐	☐☐
Have you during the past 12 months communicated with any of the services above by using the Internet?	☐	☐	☐☐

3.2 Have you over the past 12 months visited a hospital?

	Yes	No	Number of times
Hospital admission	☐	☐	☐☐
Visited an out-patient clinic:			
Psychiatric out-patient clinic	☐	☐	☐☐
Other out-patient clinics (not psychiatric department)	☐	☐	☐☐

USE OF MEDICIN

4.1 **Do you use or have you used?** *Tick once for each line.*

	Never	Now	Previously, not now	Age first time
Blood pressure lowering drugs	☐	☐	☐	
Cholesterol lowering drugs	☐	☐	☐	
Diuretics	☐	☐	☐	
Drugs for heart disease *(for example anticoagulants, antiarrhythmics, nitroglycerin)?*	☐	☐	☐	
Insulin	☐	☐	☐	
Tablets for diabetes	☐	☐	☐	
Drugs for hypothyroidism (Levaxin or thyroxine)?	☐	☐	☐	

4.2 **How often during the past four weeks have you used?** *Tick once for each line.*

	Not used in the past 4 weeks	Less than every week	Every week but not daily	Daily
Painkillers on prescription	☐	☐	☐	☐
Painkiller non-prescription	☐	☐	☐	☐
Acid suppressive medication	☐	☐	☐	☐
Sleeping pills	☐	☐	☐	☐
Tranquillizers	☐	☐	☐	☐
Antidepressants	☐	☐	☐	☐

4.3 **State the name of all medicines, both those on prescription and non-prescription drugs, you have used regularly during the last 4 weeks.** *Do not include nonprescription vitamin-, mineral- and food supplements, herbs, naturopathic remedies etc.*

If there is not enough space for all medicines, continue on a separate sheet.

DIET

5.1 **Do you usually eat breakfast every day?**

☐ No ☐ Yes

5.2 **How many units of fruit or vegetables do you eat on average per day?** *One unit is by example one apple, one salad bowl.*

Number of units

5.3 **How often do you eat these food items?** *Tick once for each line.*

	0–1 times per month	2–3 times per month	1–3 times per week	4–6 times per week	Once a day or more
Red meat (*All products from beef, mutton, pork)?*	☐	☐	☐	☐	☐
Fruits, vegetables, and berries?	☐	☐	☐	☐	☐
Lean fish *(Cod, Saithe)?*	☐	☐	☐	☐	☐
Fat fish *(salmon, trout, redfish, mackerel, herring, halibut)?*	☐	☐	☐	☐	☐

5.4 **How many glasses / containers of the following do you normally drink / eat?** *Tick once for each line.*

	Rarely / never	1–6 glasses per week	1 glass per day	2–3 glass per day	4 or more per day
Milk / Yogurt with probiotics (Biola, Cultura, Activia, Actimel, BioQ etc.)	☐	☐	☐	☐	☐
Fruit juice	☐	☐	☐	☐	☐
Soft drinks with sugar	☐	☐	☐	☐	☐
Soft drinks with artificial sweeteners	☐	☐	☐	☐	☐

5.5 **How many cups of coffee or tea do you usually drink daily?** *Put 0 for the types you <u>do not</u> drink daily.*

	Number of cups
Filtered coffee	
Boiled coffee / french plunger coffee *(coarsely ground coffee for brewing)*	
Instant coffee	
Cups of espresso-based coffee *(from coffee-machines, capsules etc.)*	
Black tea *(e.g. Earl Grey, Black currant)*	
Green tea / white tea / oolong tea	
Herbal tea *(e.g. rose hip tea, chamomile tea, Rooibos tea)*	

HEALTH ANXIETY

	Not at all	A little bit	Moderately	Quite a bit	A great deal
6.1 **Do you think there is something seriously wrong with your body?**	☐	☐	☐	☐	☐
6.2 **Do you worry a lot about your health?**	☐	☐	☐	☐	☐
6.3 **Is it hard for you to believe the doctor when he / she tells you there is nothing to worry about?**	☐	☐	☐	☐	☐
6.4 **Do you often worry about the possibility that you have a serious illness?**	☐	☐	☐	☐	☐
6.5 **If a disease is brought to your attention (e.g., on TV, radio, the internet, the newspapers, or by someone you know), do you worry about getting it yourself?**	☐	☐	☐	☐	☐
6.6 **Do you find that you are bothered by many different symptoms?**	☐	☐	☐	☐	☐
6.7 **Do you have recurring thoughts about having a disease that is difficult to be rid ofom?**	☐	☐	☐	☐	☐

PHYSICAL ACTIVITY

7.1 **If you are in paid or unpaid work, which statement describes your work best?** *Tick the most apprioate box.*

- ☐ Mostly sedentary work? *(e.g. office work, mounting))*
- ☐ Work that requires a lot of walking *(e.g. shop assistant, light industrial work, teaching)*
- ☐ Work that requires a lot of walking and lifting *(e.g. nursing, construction)*
- ☐ Heavy manual labour

7.2 **Describe your exercise and physical exertion in leisure time over the last year.** *If your activity varies throughout the year, give an average. Tick the most appropriate box.*

- ☐ Reading, watching TV / screen or other sedentary activity?
- ☐ Walking, cycling, or other forms of exercise at least 4 hours a week? (*including walking or cycling to place of work, Sunday-walking etc.)*
- ☐ Participation in recreational sports, heavy gardening, snow shoveling etc. at least 4 hours a week.
- ☐ Participation in hard training or sports competitions, regularly several times a week?

7.3 **During the last week, how much time did you spend sitting on a typical week or weekend day?** *E.g., at a desk, while visiting friends, while watching TV / screen.*

☐ Hours sitting on a weekday (both work and leisure hours*)*

☐ Hours on a weekend day

ALCOHOL

8.1 **How often do you drink alcohol??**

- ☐ Never
- ☐ Monthly or less frequently
- ☐ 2–4 times a month
- ☐ 2–3 times a week
- ☐ 4 or more times a week

8.2 **How many units of alcohol (1 beer, glass of wine or drink) do you usually drink when you drink alcohol?**

1–2	3–4	5–6	7–9	10 or more
☐	☐	☐	☐	☐

8.3 **How often do you have six or more units of alcohol in one occasion??**

- ☐ Never
- ☐ Less frequent than monthly
- ☐ Monthly
- ☐ Weekly
- ☐ Daily or almost daily

TOBACCO and SNUFF

9.1 **Do you / did you smoke daily?**

☐ Never ☐ Yes, now ☐ Yes, previously

9.2 **Have you used or do you use snuff or chewing tobacco daily?**

☐ Never ☐ Yes, now ☐ Yes, previously

QUESTIONS ABOUT CANCER

10.1 **Have you ever had**

	No	Yes	**If yes:** Age first time	**If yes:** Age last time
A mammogram	☐	☐		
Your PSA (Prostate Specific Antigen) level measured)	☐	☐		
A colon examination (colonoscopy, stool sample test)	☐	☐		

10.2 **Has anyone in your close <u>biological</u> family ever had**

	Children	Mother	Father	Maternal grandmother	Maternal grandfather	Paternal grandmother	Paternal grandfather	Aunt	Uncle	Sibling
Breast cancer	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Prostate cancer	☐		☐		☐		☐		☐	☐
Colon cancer	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

EDUCATION AND INCOME

11.1 **What is the highest levels of education you have completed?**
Tick one box only.

- ☐ Primary / partly secondary education. *(Up to 10 years of schooling)*
- ☐ Upper secondary education: *(a minimum of 3 years)*
- ☐ Tertiary education, short: College / university less than 4 years
- ☐ Tertiary education, long: College / university 4 years or more

11.2 **What was the household's total taxable income last year?**
Include income from work, social benefits and similar.

- ☐ Less than 150 000 kr
- ☐ 150 000–250 000 kr
- ☐ 251 000–350 000 kr
- ☐ 351 000–450 000 kr
- ☐ 451 000–550 000 kr
- ☐ 551 000–750 000 kr
- ☐ 751 000 –1 000 000 kr
- ☐ More than 1 000 000 kr

FAMILY AND FRIENDS

12.1 **Who do you live with?**

	Yes	No	Number
Spouse / partner	☐	☐	
Other persons over 18 years	☐	☐	
Persons under 18 years	☐	☐	

12.2 **Do you have enough friends who can give you help and support when you need it?**

☐ Yes ☐ No

12.3 **Do you have enough friends that you can talk confidentially with?**

☐ Yes ☐ No

12.4 **How often do you take part in organised gatherings, e.g., sports clubs, political meetings, religious or other associations?**

Never, or just a few times a year	1–2 times a month	Approximately once a week	More than once a week
☐	☐	☐	☐

WOMAN ONLY

13.1 **How old were you when you first started menstruating?**

Age

13.2 **Are you pregnant at the moment?**

☐ No ☐ Yes ☐ Uncertain

13.3 **How many children have you given birth to?**

Number

13.4 **If you have given birth, how many months did you breast-feed? Fill in for each child the birth year, birth weight and the number of months breast feeding.** *Fill in the best you can*

	Birth year	Birth weight in grams	Months of breastfeeding
Child 1			
Child 2			
Child 3			
Child 4			
Child 5			
Child 6			

MEN ONLY

14.1 **Have you ever had an inflammation of your prostate / urine bladder?**

☐ No ☐ Yes

14.2 **Have you ever had a vasectomy?**

☐ No ☐ Yes **If yes:** Which year was it

Thank you for your contribution.

Appendix 2.

General questionnaire Q2 in Tromsø7 2015-16.

1 STATE OF HEALTH

For every section please mark only ONE statement, which describes the state of your health TODAY.

1.1 Mobility

I have no problem walking about
I have slight problems in walking about
I have moderate problems walking about
I have severe problems walking about
I am unable to walk about

1.2 Self-Care

I have no problems washing or dressing myself
I have slight problems washing or dressing myself
I have moderate problems washing or dressing myself
I have severe problems washing or dressing myself
I am unable to wash or dress myself

1.3 Usual activities

(I.e. work, studies, household chores, family or leisure activities)

I have no problem doing my usual activities
I have slight problems doing my usual activities
I have moderate problems doing my usual activities
I have severe problems doing my usual activities
I am unable to do my usual activities

1.4 Pain/Discomfort

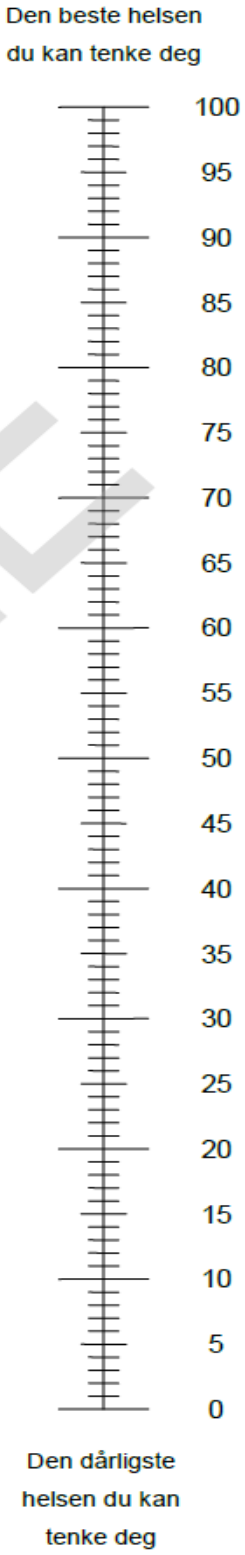
I have no pain or discomfort
I have slight pain or discomfort
I have moderate pain or discomfort
I have severe pain or discomfort
I have extreme pain or discomfort

1.5 Anxiety/Depression

I am not anxious or depressed
I am slightly anxious or depressed
I am moderately anxious or depressed
I am severely anxious or depressed
I am extremely anxious or depressed

We would like to know how good or bad your health is today. This scale is numbered from 0-100. 100 is the best health you can imagine while 0 is the worst health you can imagine. Please insert a number between 0 and 100 here.

1.6 Fill in a number between 0 and 100 which best describes your current state of health



2 CHILDHOOD/YOUTH AND AFFILIATION

2.1 Where did you live the greater part of your childhood?

(Tick once)

Tromsø

Troms, not Tromsø

Finnmark

Nordland

Norway, except Nordland, Troms, Finnmark

Abroad

2.2 How long have you lived in your current residence?

Number of years___

2.3 How was your family's financial situation during childhood?

Very good

Good

Difficult

Very difficult

2.4 What is the importance of religion in your life?

Very important

Somewhat important

No importance

2.5 What do you consider as your ethnic identity?

(Tick one or more)

Norwegian

Sami

Finnish/Kven

Other

2.6 How many siblings do you have/have you had?

Number of siblings

How many children do you have/ have you had?

Number of

2.7 Biological children
2.8 Adopted children
2.9 Step children
2.10 Foster children

2.11 Is your mother alive?
No Yes

If Yes, skip to 2.9.

If No:

2.11.1 What was your mother's age at death?
Age at death__

2.12 Is your father alive?
No Yes

If Yes, skip to 2.10.

If No:

2.12.1 What was your father's age at death?
Age at death__

What is the *highest completed education* of your parents and your domestic partner/spouse?

Primary and secondary school, up to 10 years

Vocational school/ High school minimum 3 years

College or University (Less than 4 years)

College or University (4 years or more)

2.13 Mother
2.14 Father
2.15 Spouse/domestic partner

3 WELLBEING

Below are three statements about your general wellbeing in life. Subsequently there are five statements concerning your perception and mastery of your own health. Show how much you agree or disagree with the statements by choosing the number on the rubric, which is most accurate for you.

Disagree completely Agree completely

1 2 3 4 5 6 7

3.1 In most ways my life is close to my ideal
3.2 The conditions of my life are excellent
3.3 I am satisfied with my life
3.4 I have a positive view of my future health
3.5 I can prevent serious diseases by living healthy
3.6 I know how to prevent the deterioration of my health
3.7 I can find solutions to new situations or problems with my health condition
3.8 After all, I am responsible for taking care of my health

Please indicate how well the following statements describe you or your family

Disagree completely Agree completely

1 2 3 4 5

3.9 I trust my judgments and decisions completely
3.10 I feel very happy with my family
3.11 The belief in myself gets me through difficult periods
3.12 My family is characterized by healthy cohesion
3.13 In difficult periods I have a tendency to find something good that helps me thrive and prosper
3.14 My family keeps an positive outlook on the future even in difficult periods

3.15 How would you evaluate your finances?

Very good

Good

Average

Difficult

Very difficult

4 WORK

4.1 What is your main occupation/activity?

(Tick once or more)

Works full-time

Works part-time

Housekeeping

Retired

Disability benefit recipient/work assessment allowance

Family income supplement

Unemployed

Student/military service

If Works full-time, Works part-time, Housekeeping, Retired, Student/military service, skip to 4.2

If Disability benefit recipient/work assessment allowance, Family income supplement, Unemployed:

4.1.1 For how long have you been without paid work?

3 months or less

4-6 months

7-12 months

1-2 years

3-5 years

6-9 years

10 years or more

4.2 I consider my occupation to have the following social status in society (if not currently employed, consider you latest occupation):

Very high social status

Fairly high social status

Neither high nor low social status

Fairly low social status

Very low status

If not Work full-time or Work part-time on 4.1 skip to 4.3

4.1.2 If working full-time or part-time, which of the following occupational fields describes your profession?

(Tick once)

Administrative leader or politician
Academic profession (at least 4 years of college or university education)
Work with shorter college or university education (1-3 years) and technicians
Office and customer service occupations
Sales-, service- and care professions
Agriculture, forestry or fisheries professions

Handyman, construction worker, skilled worker and the like
Process- and machine operator, transport worker or similar
Occupation with no formal educational requirements

4.1.2.1 Describe the workplace (department) where you were employed for the longest period of time the last 12 months (e.g. elementary school, hospital, bank)

Workplace: _____

4.1.2.2 Which occupation/title do/did you have at this workplace? (e.g. teacher, nurse)

Occupation: _____

4.1.4 If employed: On a scale from 0 to 10, how would you rate your job performance the past 7 days?

0 I have performed very poorly 10 I have performed excellently

If Work full-time or Work part-time on 4.1 skip to 5.1

If not Work full-time or Work part-time on 4.1:

4.1.3 Which of the following career fields best describes your last work?

(Tick once)

Administrative leader or politician
Academic profession (at least 4 years of college or university education)
Work with shorter college or university education (1-3 years) and technicians
Office and customer service occupations
Sales-, service- and care professions
Agriculture, forestry or fisheries professions
Handyman, construction worker, skilled worker and the like
Process- and machine operator, transport worker or similar
Occupation with no formal educational requirements

4.1.3.1 Describe the workplace (department) where you were employed for the longest period of time the last 12 months (e.g. elementary school, hospital, bank).

Workplace: _____

4.1.3.2 Which occupation/title do/did you have at this workplace? (e.g. teacher, nurse)

Occupation: _____

5 ILLNESS AND WORRIES

Have you had any of the following illnesses or worries?

No Yes Age first time

5.1 Have you had coronary artery bypass surgery?

5.2 Have you had percutaneous coronary intervention?

5.3 Do you have or have you had claudicatio intermittens?

If No on 5.1-5.3, skip to 5.4

If Yes on 5.1:

5.1.1 If you have had coronary artery bypass surgery - how old were you the first time?

Age first time___

If Yes on 5.2:

5.2.1 If you have had percutaneous coronary intervention - how old were you the first time?

Age first time___

If Yes on 5.3:

5.3.1 If you have had claudicatio intermittens - how old were you the first time?

Age first time___

Do you get pain in the calf when you are: No Yes

5.4 Walking?
5.5 Resting?

If No on 5.4 and 5.5, skip to 5.6:

If Yes on 5.4:

If you get pain in the calf while walking:

No Yes

5.4.1 Does the pain increase when you walk faster or uphill?
5.4.2 Does the pain disappear if you stop?

Do you get pain or discomfort in the chest when you walking?

No Yes

5.6 When walking up hills or stairs, or walking fast on level ground?
5.7 When walking at normal pace on level ground?

If No on 5.6 and 5.7, skip to 5.8.

If Yes on 5.6 or 5.7 or both:

5.6.1 If you get pain or discomfort in the chest when walking up hills or stairs, or walking fast on level ground, do you usually

Stop

Slow down

Carry on at the same pace

5.6.2 If you stop or slow down, does the pain disappear after

10 minutes or less

More than 10 minutes

5.8 Have you noticed sudden changes in your pulse or heart rhythm in the last year?

No Yes

5.9 Do you cough about daily for some periods of the year?

No Yes

If No, skip to 5.14.

If Yes:

If you cough about daily for some periods of the year

No Yes

5.9.1 Is your cough productive?

5.9.2 Have you had this kind of cough for as long as 3 months in each of the last two years?

Do you get breathless when

No Yes

5.10 Walking rapidly on level ground or up a moderate slope?
5.11 Walking calmly on level ground?
5.12 While washing or dressing?
5.13 At rest?

5.14 Do you have Crohn's disease/ulcerous colitis?

No Yes

5.15 Have you been infected with the liver virus hepatitis C?

No Yes Don't know

If No or Don't know, skip to 6.1.

If Yes:

5.15.1 Have you been treated for hepatitis C?

No Yes Don't know

6 MUSCULOSCELETAL PAIN

Have you during the last year suffered from pain and/or stiffness in muscles or joints in the following regions lasting for at least 3 consecutive months?

(Tick once for each line)

No complaint Little complaint Severe complaint

6.1 Neck, shoulder
6.2 Arms, hands
6.3 Upper part of your back
6.4 Lumbar regions
6.5 Hip, legs or feet
6.6 Other regions

Have you during the last 4 weeks suffered from pain and/or stiffness in muscles or joints in the following regions?

(Tick once for each line)

No complaint Little complaint Severe complaint

6.1 Neck, shoulder
6.2 Arms, hands
6.3 Upper part of your back
6.4 Lumbar regions
6.5 Hip, legs or feet
6.6 Other regions

7 HEADACHE

7.1 Have you been suffering from headache the last year?

No Yes

If No, skip to 8.1.

If Yes:

7.1.1 What kind of headache are you suffering from?

Migraine

Other headache

7.1.2 How many days per month do you suffer from headache?

Less than 1 day

1-6 days

7-14 days

More than 14 days

7.1.3 What is the normal intensity of your headache attacks?

Mild (do not hinder normal activity*)*

Moderate (hinder normal activity*)*

Strong *(block normal activity)*

7.1.4 What is the duration of your headache attacks generally?

Less than 4 hours

From 4 hours to 24 hours

1-3 days

More than 3 days

Is your headache generally characterized by or accompanied by

No Yes

7.1.5 Pounding/pulsatory pain?
7.1.6 Generally pressing/tightening pain?
7.1.7 Unilateral pain (left or right)?
7.1.8 Aggravated pain by physical activity?
7.1.9 Nausea and/or vomiting?
7.1.10 Light- and/or sound-sensitivity?

During or in the run up to your headache, do you have temporary:

No Yes

7.1.11 Visual disturbances?

7.1.12 Unilateral numbness in your face or hand?

7.1.13 Describe how many days you have been away from work or school during the last month due to headache?

Number of days in absence___

8 BOTHERS

8.1 How often have you been bothered by heartburn and/or acid regurgitation during the past three months?

Never Monthly Weekly Daily

If never, skip to 8.2.

If > never:

8.1.1 To what degree have you been troubled by heartburn and/or acid regurgitation?

Nothing Some A lot

8.1.2 For how long have you been troubled by heartburn and/or acid regurgitation?

Less than 3 months

3-5 months

6-12 months

More than 1 years

8.2 Did you ever fall last year?

No

Once

More than once

8.3 Are you afraid of falling?

Not at all

A bit

Very much

8.4 How have you experienced feelings of fatigue and exhaustion in the past week?

Mark on the line below the statement that most appropriate fits your experience with fatigue and exhaustion the past week

No problems with fatigue Close to a breaking point of fatigue and exhaustion

9 MEMORY

Please answer the questions below regarding your memory:

(Tick once for each line)

No Yes

9.1 Has your memory declined?
9.2 Do you often forget where you have placed your things?
9.3 Do you have difficulties finding common words in a conversation?
9.4 Do you have problems performing the daily tasks you used to master?
9.5 Have you been examined for memory problems?

If No on 9.1-9.4, skip to 10.1.

If Yes on one or more on 9.1-9.4:

9.1.1 Is your memory a problem in your daily life?

No Yes

10 INFERTILITY

10.1 Have you tried to conceive for more than one year without succeeding in becoming pregnant?

No Yes

If No, skip to 11.1.

If Yes:

If you have tried to conceive for more than one year without succeeding in becoming pregnant

No Yes Don't know

10.1.1 Was this due to conditions with yourself?
10.1.2 Was this due to conditions with your partner?
10.1.3 Have you received medical treatment for childlessness?

No Yes

If No, skip to 11.1.

If Yes:

If you or your partner has received treatment for childlessness, what type of treatment have you received?

Number of times

10.1.3.1 Have you received treatment for stimulation of ovulation?
10.1.3.2 Have you received treatment for stimulation of ovulation followed by artificial insemination (not husband/partner)?
10.1.3.3 Have you received artificial insemination (not husband/partner)?
10.1.3.4 Have you received invitrofertilisation (IVF/ICSI)?
10.1.3.5 Have you received other treatment modalities for childlessness?

How many children have you got in infertility treatment:

Number of children

10.1.3.6 At the university hospital of Northern Norway?
10.1.3.7 At other treatment institutions in Norway?
10.1.3.8 Abroad?

11 FAMILY AND FRIENDS

Tick the relatives which has or have had the following disease(s)

Mother Father Children Sibling(s) None of the closest relatives

11.1 Heart attack before the age of 60
11.2 Angina pectoris (Heart cramp)
11.3 Brain hemorrhage
11.4 Asthma
11.5 Diabetes
11.6 Psychological problems
11.7 Problems with substance abuse

12 SLEEP

How many days per week

(Tick the number of days) Number for days a week 0 1 2 3 4 5 6 7

12.1 Do you usually use more than 30 minutes to fall asleep?
12.2 Do you usually wake up for more than 30 minutes in the middle of the night?
12.3 Do you wake up 30 minutes earlier than you wished, without being able to go back to sleep again?
12.4 Have you not felt refreshed after sleep?
12.5 Felt so tired that it has affected your work, school or private life?
12.6 Have you been dissatisfied with your sleep?

12.7 If sleep problems, how long time?

Less than 1 week

1-3 weeks

1 months

2 months

3 months

4-6 months

7-12 months

1-5 years

6-10 years

More than 10 years

Do not have sleep problems

12.8 Do you usually work shifts or at night?

No Yes

When do you usually go to sleep?

12.9 On work days

00:30-24:00 (dropdown menu)

12.10 On non-work days

00:30-24:00 (dropdown menu)

For how long are you awake before you fall asleep?

12.11 On work days

Minutes_____

12.12 On non-work days

Minutes_____

What time do you usually wake up?

12.13 During work week

kl. 00:30-24:00 (dropdown menu)

12.14 During free days

kl. 00:30-24:00 (dropdown menu)

12.15 How often do you take naps at daytime?

Never or less often than once per month

Less than once per week

1-2 days per week

3-5 days per week

Every day or almost every day

If Never or less than once per month, skip to 12.16

If>Never or less than once per month:

12.15.1 If you take a nap, how long does it usually last?

Minutes___

12.16 Do you snore while sleeping?

Never or less than once per month

Less than once per week

1-2 nights per week

3-5 nights per week

Daily or almost daily

Don't know

12.17 Have you had breathing pauses (sleep apnea) at sleep?

Never or less than once per month

Less than once per week

1-2 nights per week

3-5 nights per week

Daily or almost daily

Don't know

How likely are you to doze off or fall asleep in the following situation?

Use the scale from 0-3 for each situation:

0= No chance of dosing

1= Slight chance of dozing

2= Moderate chance of dozing

3= High chance of dozing

Situation Likelihood of dozing off or fall asleep (0-3)

12.18 Sitting and reading
12.19 Watching TV
12.20 Sitting inactive in a public place (e.g. a theatre or meeting)
12.21 As a passenger in a car for an hour without break
12.22 Lying down to rest in the afternoon when circumstances permit
12.23 When you are sitting and talking to someone
12.24 When you are sitting quietly after lunch without alcohol
12.25 When you are in a car, while stopped for a few minutes in traffic

14 ABDOMEN

14.1 In the last 3 months, how often did you have discomfort or pain anywhere in your abdomen?

Never

Less than one day per month

One day a month

Two or three days a month

One day a week

More than one day a week

Every day

If Never, skip to 14.2.

If > Never and male, skip to 14.1.2.

If >Never and female:

PAIN IN ABDOMEN

14.1.1 Did this discomfort or pain occur only during your menstrual bleeding and not at other times?

No Yes I have not had menstrual bleedings the last 3 months

If Yes, skip to 14.2.

If No or I have not had menstrual bleedings the last 3 months:

PAIN IN ABDOMEN

14.1.2 Have you had this discomfort or pain 6 months or longer?

No Yes

If No, skip to 14.2.

If Yes:

PAIN IN ABDOMEN

Never or rarely Sometimes Often Most of the time Always

14.1.2.1 How often did this discomfort or pain get better or stop after you had a bowel movement?
14.1.2.2 When discomfort or pain started, did you have more frequent bowel movements?
14.1.2.3 When discomfort or pain started, did you have less frequent bowel movements?
14.1.2.4 When discomfort or pain started, were your stools (bowel movements) looser?
14.1.2.5 When discomfort or pain started, how often did you have harder stools?
14.1.2.6 In the last 3 months, how often did you have hard or lumpy stools?
14.1.2.7 In the last 3 months, how often did you have loose, mushy or watery stools?

14.2 How often do you usually have a bowel movement?

4 times a day or more often

1-3 times per day

4-6 times per week

1-3 times per week

Less than once per week

15 USE OF HEALTH SERVICES

15.1 For how long have you been going to your present GP?

Less than 1 year

1-2 years

3-4 years

More than 4 years

15.2 Did you have an appointment with your GP during the last 12 months?

No Yes

If No, skip to 15.3.

If Yes:

15.2.1 During your last appointment were you referred to

(Tick once or more)

Physiotherapist

Chiropractor

Psychiatrist/psychologist

Radiological investigation / imaging

Hospital outpatient clinic

Private practicing secondary care specialist

Other kinds of referral

Not referred

15.2.2 During the last 12 months, did you ask or want to be referred to radiological investigation or secondary care specialist, but was not referred by your GP?

Yes, once

Yes, several times

No, I was referred when I asked or wanted to be referred

No, referral was not relevant

If No, I was referred when I asked or wanted to be referred or No, referral was not relevant, skip to 15.3

If Yes:

15.2.2.1 The fact that you were not referred, did it affect your health in any way?

No Yes, temporarily Yes, permanent consequences

How often during the last year have you used the following internet-services for information and advice on health and disease issues?

Never Once A few times Often

15.3 Applications ("Apps") for smart phone or tablet

15.4 Search engines (like Google)

15.5 Social media (like Facebook or similar)

15.6 Video services (Like Youtube)

If Never on the entire previous, skip to 16.1.

If >Never:

Based on the information you found on the Internet – have you?

Never Once A few times Often

15.3.1 Decided to go to a doctor?
15.3.2 Decided not to go to a doctor?
15.3.3 Discussed the information with a doctor?
15.3.4 Changed your medication without consultation with a doctor?
15.3.5 Have you been unsure your diagnosis is correct?
15.3.6 Have you been unsure whether the treatment you have received is correct?
15.3.7 Have you decided to seek out complementary or alternative treatment?
15.3.8 Have you made lifestyle changes?
15.3.9 Have you felt anxiety?
15.3.10 Felt reassured?
15.3.11 Felt more knowledgeable?
15.3.12 Felt more confused?

16 COMPLIMENTARY AND ALTERNATIVE MEDICINE

16.1 Have you used herbal medicines during the last 12 months?

No Yes

16.2 Have you used meditation, yoga, qi gong or Tai Chi as self-treatment during the last 12 months?

No Yes

17 PAINKILLERS AND ANTIINFLAMMATORIC MEDICINES

17.1 Have you used analgesics and anti-inflammatory medication regularly in the past year? (i.e. acetylsalicylic acid, paracetamol, ibuprofen, diclofenac, naproxen)?

These include both over-the-counter and prescription only medicines, also including acetylsalicylic acid, which is used in low dosage as a blood thinning drug.

No Yes

If No, skip to 18.1.

If Yes:

Which analgesics and anti-inflammatory medication have your used the past year?

(Tick one or more)

17.1.1 «Baby» or low dose of Acetylsalicylic acid

(75 mg or 160 mg per tablet, i.e. Acetylsalicylic acid® i.e Albyl-E® Asasantin Retard®)

No Yes

17.1.2 Acetylsalicylic acid high dosage

(300-500 mg acetylsalicylic acid per tablet, i.e. Aspirin® Dispril® Globoid®)

No Yes

17.1.3 Paracetamol

(i.e. Pamol® Panodil® Paracet® Paracetamol® Pinex® Paracetduo®)

No Yes

17.1.4 Acetaminophen combined with med codeine phosphate/tramadol

(i.e. Paralgin forte® Codaxol® Paralgin major® Paralgin minor® Paramax Comp Vitabalans® Pinex Forte® Pinex Major® Trampalgin®)

No Yes

17.1.5 Fenazon

(i.e. Fanalgin® Fenazon-koffein® Fenazon-koffein sterke®)

No Yes

17.1.6 Ibuprofen etc.

(i.e. Brufen Retard® Burana® Ibumax® Ibumetin® Ibuprofen Ibuprox® Ibux® Orudis® Seractiv® Ketesse® Orodek®)

No Yes

17.1.7 Diclofenac etc.

(i.e. Cataflam® Diclofenac® DiclofenacKalium® Modifenac® Voltaren® Voltarol® Arthrotec® Toradol®)

No Yes

17.1.8 Naproxen

(i.e. Napren-E® Naproxen-E® Naproxen® Vimovo®)

No Yes

17.1.9 Other analgesics and anti-inflammatory medication

(I.e. Brexidol® Piroxicam® Meloxicam® Migea® Celebra® Dynastat® Arcoxia® Relifex®)

No Yes

If No on 17.1.1, skip to 18.1.

If Yes on 17.1.1:

17.1.1.1 Have you used "Baby" or low dose acetylsalicylic acid weekly or more often the past year?

No Yes

If No, skip to 18.1.

If Yes:

If you have used "Baby" or low dose acetylsalicylic acid regularly in the past year

17.1.1.1.1 How many days a week?

1

2-3

4-5

6+ days

17.1.1.1.2 How many tablets per week?

1-2

3-5

6-14

15+

17.1.1.1.3 For how long have you had this regular use?

Number of years__

If No on 17.1.2, skip to 18.1.

If Yes on 17.1.2:

17.1.2.1 Have you used high dose acetylsalicylic acid weekly or more often the past year?

No Yes

If No, skip to 18.1.

If Yes:

If you have used high dose acetylsalicylic acid weekly or more often the past year

17.1.2.1.1 How many days per week?

1

2-3

4-5

6+ days

17.1.2.1.2 How many tablets in total per week?

1-2

3-5

6-14

15+

17.1.2.1.3 For how long have you had this regular use?

Number of years__

If No on 17.1.3, skip to 18.1.

If Yes on 17.1.3:

17.1. 3.1 Have you used paracetamol weekly or more often the past year?

No Yes

If No, skip to 18.1.

If Yes:

If you have used paracetamol weekly or more often the past year

17.1.3.1.1 How many days per week?

1

2-3

4-5

6+ days

17.1.3.1.2 How many tablets in total per week?

1-2

3-5

6-14

15+

17.1.3.1.3 For how long have you had this regular use?

Number of years__

If No on 17.1.4, skip to 18.1.

If Yes on 17.1.4:

17.1.4.1 Have you used acetaminophen combined with med codeine phosphate /tramadol weekly or more often the past year?

No Yes

If No, skip to 18.1.

If Yes:

If you have used acetaminophen combined with med codeine phosphate /tramadol weekly or more often the past year

17.1.4.1.1 How many days per week?

1

2-3

4-5

6+ days

17.1.4.1.2 How many tablets in total per week?

1-2

3-5

6-14

15+

17.1.4.1.3 For how long have you had this regular use?

Number of years__

If No on 17.1.5, skip to 18.1.

If Yes on 17.1.5:

17.1.5.1 Have you used fenazon weekly or more often the past year?

No Yes

If No, skip to 18.1.

If Yes:

If you have used fenazon weekly or more often the past year

17.1.5.1.1 How many days per week?

1

2-3

4-5

6+ days

17.1.5.1.2 How many tablets in total per week?

1-2

3-5

6-14

15+

17.1.5.1.3 For how long have you had this regular use?

Number of years__

If No on 17.1.6, skip to 18.1.

If Yes on 17.1.6:

17.1.6.1 Have you used ibuprofen etc. weekly or more often the past year?

No Yes

If No, skip to 18.1.

If Yes:

If you have used ibuprofen etc. weekly or more often the past year

17.1.6.1.1 How many days per week?

1

2-3

4-5

6+ days

17.1.6.1.2 How many tablets in total per week?

1-2

3-5

6-14

15+

17.1.6.1.3 For how long have you had this regular use?

Number of years__

If No on 17.1.7, skip to 18.1.

If Yes on 17.1.7:

17.1.7.1 Have you used diclofenac etc. weekly or more often the past year?

No Yes

If No, skip to 18.1.

If Yes:

If you have used diclofenac etc. weekly or more often the past year.

17.1.7.1.1 How many days per week?

1

2-3

4-5

6+ days

17.1.7.1.2 How many tablets in total per week?

1-2

3-5

6-14

15+

17.1.7.1.3 For how long have you had this regular use?

Number of years__

If No on 17.1.8, skip to 18.1.

If Yes on 17.1.8:

17.1.8.1 Have you used naproxen weekly or more often the past year?

No Yes

If No, skip to 18.1.

If Yes:

If you have used naproxen weekly or more often the past year?

17.1.8.1.1 How many days per week?

1

2-3

4-5

6+ days

17.1.8.1.2 How many tablets in total per week?

1-2

3-5

6-14

15+

17.1.8.1.3 For how long have you had this regular use?

Number of years__

If No on 17.1.9, skip to 18.1.

If Yes on 17.1.9:

17.1.9.1 Have you used other analgesics and anti-inflammatory medication weekly or more often the past year?

No Yes

If No, skip to 18.1.

If Yes:

If you have used other analgesics and anti-inflammatory medication weekly or more often the past year

17.1.9.1.1 How many days per week?

1

2-3

4-5

6+ days

17.1.9.1.2 How many tablets in total per week?

1-2

3-5

6-14

15+

17.1.9.1.3 For how long have you had this regular use?

Number of years__

18 MEDICINE INFORMATION

18.1 Have you used medicines (nonprescription and prescription) regularly during the last 4 weeks?

Do not include dietary supplements (vitamins, minerals, omega-3, herbs or other natural remedies)

No Yes

If No, skip to 19.1.

If Yes:

Think about the information you receive about your medicines from your general practitioner. Indicate whether you agree that you receive the following information

I receive information about… Strongly disagree Disagree Neutral Agree Strongly agree

18.1.1 The reason why I should take my medicines.
18.1.2 How I should take my medicines.
18.1.3 Which side effects I should be aware of.
18.1.4 How other medicines and/or food will/do influence my medicines.

Think about the information you receive about your medicines from your pharmacy. Indicate whether you agree that you receive the following information:

I receive information about… Strongly disagree Disagree Neutral Agree Strongly agree

18.1.5 The reason why I should take my medicines.
18.1.6 How I should take my medicines.
18.1.7 Which side effects I should be aware of.
18.1.8 How other medicines and/or food will/do influence my medicines.

18.1.9 Do you have help with your medicines?

No Yes

18.1.10 Regardless of whether you have help with your medicines or not, do you need more help?

I do not need any more help

I would like some more help

I would like a lot more help

18.1.11 Do you need more information about your medicines?

I do not want any more information

I would like some more information

I would like a lot more information

18.1.12 Generally, how would you describe the importance of your medicines?

Not important at all

Not very important

Important

Very important

18.1.13 Are you concerned about your medicines?

Not at all concerned

Concerned

Very concerned

If Not at all concerned, skip to 18.1.13.

If Concerned or Very concerned:

18.1.13.1 Please indicate which concerns you have about your medicines

(Tick once or more)

I am concerned about..

The long-term effects of these medicines

That these medicines would give me unpleasant side effects

That these medicines will disrupt my life

I will not be able to take my medicines correctly

Becoming too dependent on these medicines

That the medicines will become less effective if used regularly

That the medicines do more harm than good

The cost of my medicines

Other

Either because of forgetfulness, inconvenience or because they do not want to, it is common that people not always take the medicine they have been prescribed. The following questions concern your habits when taking *your* medicine.

18.1.14 How many times a week do you forget to take your medicines?

Less than once a week

Once a week

2-4 times a week

5 times a week or more

18.1.15 How many times a week do you decide to miss out your medicines?

Less than once a week

Once a week

2-4 times a week

5 times a week or more

19 PHYSICAL ACTIVITY

19.1 How often do you exercise?

(i.e. walking, skiing, swimming or training/sports)

Never

Less than once a week

Once a week

2-3 times a week

Approximately every day

If Never, skip to 20.1.

If >Never:

19.1.1 If you exercise - how hard do you exercise?

Easy - you do not become shortwinded or sweaty

You become shortwinded and sweaty

Hard - you become exhausted

19.1.2 For how long time do you exercise? (give an average)

Less than 15 minutes

15-29 minutes

30-60 minutes

More than 1 hour

20 FOOD HABITS

How often do you usually eat?

Tick once for each line

0-1 times per month 2-3 times per month 1-3 times per week More than 3 times per week

20.1 Fresh water fish (not farmed)
20.2 Salt water fish (not farmed)
20.3 Farmed fish (salmon, trout, char)
20.4 Tuna fish (fresh or canned)
20.5 Fish bread spread
20.6 Mussels, shells
20.7 Brown content in crabs
20.8 Meat from whale or seal
20.9 Pluck (liver/kidney/heart) from reindeer or elk/moose
20.10 Pluck (liver/kidney/heart) from ptarmigan/grouse
20.11 Tomatoes and tomato-based products (e.g. tomato, ketchup)

How many times per year do/did you usually eat

In adulthood: times per year In childhood: times per year

20.12 "Mølje" (cod or pollack meat, liver, and roe)
20.13 Seagulls' egg
20.14 Reindeer meat
20.15 Elk meat
20.16 Wild mushroom and wild berries (blueberries/lingonberries/cloudberries)

Do you use the following food supplements?

(Tick once for each line)

No Sometimes Daily during the winter season Daily

20.17 Cod liver oil or cod liver oil capsules
20.18 Omega 3 capsules (fish oil, seal oil)
20.19 Calsium tablets
20.20 Vitamin supplement with vitamin D

No Sometimes Only while travelling Daily

20.21 Milk acid bacteria/probiotics

21 LIGHT/SUN EXPOSURE

21.1 Have you been on holiday in the sun during the last two months?

No Yes

21.2 Do you use a solarium?

Yes, weekly Yes, sometimes Never

22 WEIGHT

22.1 What weight would you be satisfied with (your "ideal" weight)

(kg)___

22.2 Are you satisfied with your present body weight?

Yes No

22.3 Estimate your body weight when you were 25 years old

(kg)___

22.4 Have you involuntary lost weight during the last 6 months?

Yes No

If No, skip to 23.1 or 24.1 depending on your sex.

If Yes:

22.4.1 If you have involuntary lost weight during the last 6 months, how many kilos?

(kg)___

23 MENS HEALTH

23.1 Have you experienced any problems achieving an erection within the past 3 months?

No Yes

If No, skip to 23.2.

If Yes:

23.1.1 How do you rate your confidence that you could get and keep an erection?

Very low Low Moderate High Very high

23.1.2 When you had erections with sexual stimulation, how often were your erections hard enough for penetration?

Almost never or never A few times (much less than half the time) Sometimes (about half the time) Most times (Much more than half the time) Almost always or always

23.1.3 During sexual intercourse, how often were you able to maintain your erection after you had penetrated your partner?

Almost never or never A few times (much less than half the time) Sometimes (about half the time) Most times (Much more than half the time) Almost always or always

23.1.4 During sexual intercourse, how difficult was it to maintain your erection to completion of intercourse?

Extremely difficult Very difficult Difficult Slightly difficult Not difficult

23.1.5 When you attempted sexual intercourse, how often was it satisfactory for you?

Almost never or never A few times (much less than half the time) Sometimes (about half the time) Most times (Much more than half the time) Almost always or always

23.2 Have any of your partners had a spontaneous abortion after becoming pregnant with you?

No Yes Don't know

If No or Don't know, skip to 25.1.

If Yes:

23.2.1 In total, how many spontaneous abortions have your partner(s) had after being pregnant with you?

Number of times__

24 WOMENS HEALTH

24.1 Have you ever had a spontaneous abortion?

No Yes Don't know

If No or Don't know, skip to 24.2.

If Yes:

24.1.1 How many spontaneous abortions have you had?

Number of times__

MENSTURATION

24.2 Do you still menstruate?

No Yes

If Yes, skip to 24.3.

If No:

24.2.1 If you do not have natural menstruation, why did it stop?

(Tick once)

It stopped by itself

Uterus surgery

Both Ovaries were removed

Hormonal intrauterine device

Other reason (e.g. radiation, chemo therapy)

24.2.2 If you do not have natural menstruation, how old were you when it stopped?

Age ___

24.3 Have you during your lifetime been bothered by menstrual pain?

No Yes

If No, skip to 24.4.

If Yes on 24.3 + No on 24.2:

How old were you when the pain was most bothersome?

24.3.1 from age_

24.3.2 to age _

24.3.3 How long did the pain usually last at that time?

Less than one day

1 day

2 days

3 days

4 days

More than 4 days

24.3.4 On a scale from 0 to 10, where 0 is no pain at all, while 10 is the most severe pain imaginable, how strong was the pain usually at that time?

No pain at all 0 1 2 3 4 5 6 7 8 9 10 Most severe pain imaginable

24.3.5 Were you sometimes away from school or work because of menstrual pain?

Never Sometimes Often Most of the time

If Yes on 24.3 + Yes on 24.2:

24.3.6 If you still menstruate - How long does the pain usually last?

Less than one day

1 day

2 days

3 days

4 days

More than 4 days

24.3.4 On a scale from 0 to 10, where 0 is no pain at all while 10 is the most severe pain imaginable, how strong was the pain usually at that time?

No pain at all 0 1 2 3 4 5 6 7 8 9 10 Most severe pain imaginable

24.3.8 Are you sometimes away from school or work because of menstrual pain?

Never Sometimes Often Most of the time

HORMONAL CONTRACEPTIVES

24.4 Do you use now, or have you ever used hormonal contraceptives (incl. oral contraceptive pills, mini-pills, birth control patch, intrauterine birth control products, and implants)?

No Yes, previously Yes, now

If No, skip to 24.5

If Yes, previously on 24.4:

24.4.1 Age first time you used hormonal contraceptives

Age first time use __

24.4.2 Age last time you used hormonal contraceptives

Alder last time use __

24.4.3 In total, how many years have you used hormonal contraceptives?

Number of years__

If Yes, now on 24.4:

24.4.1 Age first time you used hormonal contraceptives

Age first time use __

24.4.3 In total, how many years have you used hormonal contraceptives?

Number of years__

24.4.5 If you use hormonal contraceptives now - which brand do you currently use?

Oral contraceptive pills (Loette Microgynon Oralcon Marvelon Mercilon Yasmin Yasminelle Yaz Zoely Synfase Qlaira)

Mini-pills (Conludag Cerazette)

Birth control patch (Evra)

Implants (Depo-Provera, Nexplanon)

Intrauterine birth control products, implants (Jaydess, Mirena, NuvaRing)

USE OF SEX HORMONES

24.5 Do you use now or have ever used estrogens to treat climacteric problems or for any other reason (tablets/patches/vaginal ring/tablets/creams)?

No Yes, previously Yes, now

If No, skip to 24.6.

If Yes, previously 24.5:

24.5.1 Age first time you used estrogens

Age first time use __

24.5.2 Age last time you used estrogens

Age last time use __

24.5.3 In total, how many years have you used estrogens?

Number of years__

If Yes, now on 24.5:

24.5.1 Age first time you used estrogens

Age first time use __

24.5.3 In total, how many years have you used estrogens?

Number of years__

24.5.4 If you use estrogens now- which brand do you use?

Patches (Evorel Estalis Estradot Sequidot)

Tablets (Activelle Cliovelle Eviana Indivina Novofem Trisekvens Progynova Livial)

Ovesterin tablets

Vaginal ring/Vaginal tablet (Estring Vagifem)

Ovesterin vaginal crème, Ovesterin vagitories

24.6 Did you or do you have hot flushes during the climacteric period?

Yes, definitely Yes, sometimes No, not much No, not at all

24.7 Did you or do you suffer from night sweats during the climacteric period?

Yes, definitely Yes, sometimes No, not much No, not at all

VAGINAL BULGE

24.8 Can you feel a bulge from or in the vagina?

Genital prolapse is a condition you can feel from or in the vagina.

Not at all

A little

Moderately

A lot

If Not at all, skip to 25.1.

If A little, Moderately or A lot:

VAGINAL BULGE

Not at all A little Moderately A lot

24.8.1 Do you have a bulge in your vagina that worsens during the course of the day?
24.8.2 How much do you think your prolapse problem affects your life?
24.8.3 Does your prolapse affect your physical activities (e.g. going for a walk, run, sport, gym etc.)?
24.8.4 Do you have to strain in order to empty your bladder?
24.8.5 Does your prolapse affect your sexual life? (a vaginal bulge which gets in the way of sex)

24.8.6 If necessary, can you push up the prolapse with your fingers?

Never

Occasionally

Often

Always

24.8.7 Do you feel completely empty after having had defecation?

Always

Most of the time

Quite often

Usually not

24.8.8 Have you visited a medical doctor for your prolapse?

No Yes

25 SEXUAL HEALTH

25.1 Have you been sexually active during the last 12 months?

No Yes

25.2 What is your total number of sexual partners? (Put in 0 if you have not had a sex partner)

Number of sexual partners __

25.3 Have you ever practised oral sex? (performed on partner and/or received)

No Yes

If No, skip to 26.1.

If Yes:

25.3.1 Have you practised oral sex during the last 12 months?

No Yes

26 URINATING PROBLEMS

26.1 Have you experienced problems urinating the past month?

(i.e. weak urinary stream, a sensation of not being able to empty your bladder or abnormal high frequency of bathroom visits)

No Yes

If No, skip to 27.1.

If Yes:

URINATING PROBLEMS

Not at all Less than 1 in 5 times Less than half the time About half the time More than half the time Almost always

26.1.1 How often have you had the sensation of not emptying your bladder?
26.1.2 How often have you had to urinate less than every two hours?
26.1.3 How often have you found you stopped and started again several times when you urinated?
26.1.4 How often have you found it difficult to postpone urination?
26.1.5 How often have you had a weak urinary stream?
26.1.6 How often have you had to strain to start urination?
26.1.7 How many times did you typically get up at night to urinate?

27 INCONTINENCE

27.1 How often do you leak urine?
(Tick once)

Never

About once a week or less often

Two or three times a week

About once a day

Several times a day

All the time

If Never, skip to 28.1.

If >Never:

URINARY INCONTINENCE

27.1.1 How much urine do you usually leak?
(Tick once)

None

A small amount

A moderate amount

A large amount

27.1.2 Overall, how much does leaking urine interfere with your everyday life?
Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 A great deal

27.1.3 When does urine leak?
(Tick once or more)

Never – urine does not leak

Leaks before you can get to the toilet.

Leaks when you cough or sneeze.

Leaks when you are asleep.

Leaks when you are physically active/exercising.

Leaks when you have finished urinating and are dressed.

Leaks for no obvious reason.

Leaks all the time.

27.1.4 Have you sought any help for urinary incontinence

No Yes

28 ANXIETY AND DEPRESSION

Below you find a list of different situations. Have you experienced some of them in the last week (including today)?

(Tick once for each complaint)

	No complaint	Little complaint	Pretty much	Very much

28.1 Sudden fear without apparent reason
28. Felt afraid or anxious
28.3 Faintness or dizziness
28.4 Felt tense or upset
28.5 Easily blamed yourself
28.6 Sleeping problems
28.7 Depressed or sad
28.8 Felt useless, worthless
28.9 Felt that everything is a struggle
28.10 Felt hopelessness with regard to the future

Tick the box beside the reply that is closest to how you have been feeling in the past week. Don't think too long over your replies: your immediate is best.

28.11 I feel tense or 'wound up'.

Most of the time

A lot of the time

From time to time, occasionally

Not at all

28.12 I still enjoy the things I used to enjoy.

Definitely as much

Not quite so much

Only a little

Hardly at all

28.13 I get a sort of frightened feeling as if something awful is about to happen.

Very definitely and quite badly

Yes, but not too badly

A little but it doesn’t worry me

Not at all

28.14 I can laugh and see the funny side of things.

As much as I always could

Not quite as much now

Definitely not so much now

Not at all

28.15 Worrying thoughts go through my mind.

A great deal of the time

A lot of the time

From time to time, but not too often

Only occasionally

28.16 I feel cheerful.

Not at all

Not often

Sometimes

Most of the time

28.I can sit at ease and feel relaxed.

Definitely

Usually

Not often

Not at all

28.18 I feel as if I am slowed down.

Nearly all the time

Very often

Sometimes

Not at all

28.19 I get a sort of frightened feeling like 'butterflies' in the stomach.

Not at all

Occasionally

Quite often

Very often

28.20 I have lost interest in my appearance.

Definitely

I don’t take as much care as I should

I may not take as much as ever

I take just as much care as ever

28.21 I feel restless as I have to be on the move.

Very much indeed

Quite a lot

Not very much

Not at all

28.22 I look forward with enjoyment to things.

As much as I ever did

Rather less than I used to

Definitely less than I used to

Hardly at all

28.23 I get sudden feelings of panic.

Very much indeed

Quite often

Not very often

Not at all

28.24 I can enjoy a good book or radio or TV program.

Often

Sometimes

Not often

Very seldom

29 WORRIES

Specify how typical or characteristic the following statement is for you

(Tick once for each statement, from 1=Not typical to 5= Very typical)

Not typical	Somewhat typical			Very typical
1	2	3	4	5

29.1 I worry all the time

29.2 Many situations make me worry

29.3 I am always worrying about something

30 Mental Trauma

Have you ever experienced one of the following events?

(Tick once or more for each line)

	No, have not experienced	Yes, before 18 years old	Yes, after 18 years old	Yes, the last year
30.1 A life-threatening illness or a serious accident (for example, fire, work accident, or car accident)				
30.2 Been exposed to violence (for example, hit, kicked, beaten, robbed, or threatened with a firearm)				
30.3 Been exposed to sexual abuse, i. e. sexual actions against your will				
30.4 Been called negative things, marginalized, threatened or bullied by schoolmates, fellow students, or coworkers over a longer period				
30.5 Witnessed someone close to you being exposed to violence or sexual abuse (for example, hit, kicked, beaten, robbed, or threatened with a firearm				
30.6 Experienced something else that was frightening, dangerous, or violent (for example, natural disaster, war, terror attack, held captive)				
30.7 Death of a close one and difficulty accepting the loss, yearning for the deceased, and intense emotional pain related to the loss.				
30.8 Received painful medical treatment when in hospital due to sickness or serious injury.				
30.9 Received painful medical treatmentat at the dentist.				
30.10 That someone close to you had a life-threatening illness or was exposed to a serious accident.				
30.11 Failure of care in childhood, i.e. not having received the necessary of food, clothing, protection and care/love from parents/caregivers				

No Yes

If No on all 30.1-30.11, skip to 31.1

If Yes on at least one of 30.1-30.11:

30.1.1 Do you still think a lot about what happened?

No Yes

31 ALCOHOL

31.1 Have you been drinking alcoholic beverages during the last year?

No Yes

If No, skip to 31.1.1.15.

If Yes:

As exact as you can, give an estimate of your alcohol habits. Keep the past year in mind when filling in.

31.1.1-*31.1.8.2*

9. Alkoholholdige drikker

Svar enten pr. måned eller pr. uke. Merk at porsjonsenhetene er forskjellige, 1/5 liter tilsvarer ett glass (2 dl), mens 1/3 liter tilsvarer 0,33 liter glassflaske/boks.

	Gang pr. måned			eller	Gang pr. uke					Mengde pr. gang						
	Aldri/ sjelden	1	2	3	1	2-3	4-5	6-7								
Øl, sterk øl, pils	☐	☐	☐	☐	☐	☐	☐	☐	(liter)	1/3 ☐	½ ☐	1 ☐	2 ☐	3 ☐	4+ ☐	
Lettøl	☐	☐	☐	☐	☐	☐	☐	☐	(liter)	1/3 ☐	½ ☐	1 ☐	2 ☐	3 ☐	4+ ☐	
Rusbrus, Cider m/alkohol	☐	☐	☐	☐	☐	☐	☐	☐	(liter)	1/5 ☐	1/3 ☐	½ ☐	1 ☐	1½ ☐	2+ ☐	
Rødvin	☐	☐	☐	☐	☐	☐	☐	☐	(vinglass)	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6+ ☐	
Hvitvin	☐	☐	☐	☐	☐	☐	☐	☐	(vinglass)	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6+ ☐	
Hetvin (portvin, sherry o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	(1 glass = 4cl)	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6+ ☐	
Brennevin, likør	☐	☐	☐	☐	☐	☐	☐	☐	(1 dram = 4cl)	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6+ ☐	
Blandede drinker, cocktail	☐	☐	☐	☐	☐	☐	☐	☐	(drink)	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6+ ☐	

How often the past year have you:

Never Less than monthly Monthly Weekly Daily

31.1.1.9 Not been able to stop drinking alcohol when first started?
31.1.1.10 Failed to do what was normally expected from you because of drinking?
31.1.1.11 Needed alcohol in the morning to get yourself going after a heavy drinking session?
31.1.1.12 Had a feeling of guilt or remorse after drinking?
31.1.1.13 Been unable to remember what happened the night before because you had been drinking?
31.1.1.14 Drunk so much that you felt highly intoxicated (drunk)?

How often the past year have you:

Never Yes, but not during the past year Yes, during the past year

31.1.1.15 Have you or someone else been injured because of your drinking?
31.1.1.16 Has a relative or friend or a doctor or another health worker been concerned about your drinking or suggested you cut down?

32 DRUG USE

32.1 Do/did you use drugs other than alcohol (for example cannabis, amphetamines, cocaine, heroin, hallucinogens, solvents, GHB)?

No Yes, now Yes, previously

If No skip to 32.2.

If Yes, now Yes, previously:

32.1.1 If you do/did use drugs other than alcohol (for example cannabis, amphetamines, cocaine, heroin, hallucinogens, solvents, GHB) - how old were you the first time?

Age first time ___

32.1.2 During the last 12 months, how often did you use other drugs than alcohol (for example cannabis, amphetamines, cocaine, heroin, hallucinogens, solvents, GHB)?

No use the last 12 months

Once a month or less often

2-4 times a month

2-3 times a week

4 times a week or more often

32.2 Do/did you use prescription drugs for recreational use (for example calming pills, sleeping pills, pain-relievers, ADHD-medication)?

No Yes, now Yes, previously

If No skip to 33.1.

If Yes, now or Yes, previously

32.2.1 If you do/did use prescription drugs for recreational use (for example calming pills, sleeping pills, pain-relievers, ADHD-medication) - how old were you the first time?

Age first time use ___

32.2.2 During the last 12 months, how often did you use prescription drugs for recreational use (for example calming pills, sleeping pills, pain-relievers, ADHD-medication)?

No use last 12 months

Once a month or less often

2-4 times a month

2-3 times a week

4 times a week or more often

33 SMOKE AND SNUFF

DAILY SMOKING

33.1 Do you/did you smoke daily?

Never Yes, now Yes, previously

If Never, skip to 33.2

If Yes, now or Yes, previously

DAILY SMOKING

33.1.1 How old were you when you began smoking daily?

Age__

33.1.2 How many years in all have you smoked daily?

Number of years__

33.1.3 How many cigarettes do you or did you usually smoke per day?

Number of cigarettes ____

If Yes, previously on 33.1:

33.1.4 If you previously smoked daily, how long is it since you stopped?

Number of years__

OCCASIONAL SMOKING

33.2 Do you smoke, or have you smoked sometimes, but not daily?

Never Yes, now Yes, previously

If Never, skip to 33.3.

If Yes, now or Yes, previously:

OCCASIONAL SMOKING

33.2.1 How many cigarettes do you or did you usually smoke per day?

Number of cigarettes__

DAILY USE OF SNUFF

33.3 Have you used or do you use snuff or chewing tobacco?

Never Yes, now Yes, previously

If Never, skip to 33.4.

If Yes, now or Yes, previously?

DAILY USE OF SNUFF

33.3.1 How old were you when you began using snuff or chewing tobacco?

Age__

33.3.2 How many years in all have you used snuff or chewing tobacco?

Number of years____

33.3.3 If you use or have used snuff - how many portions do/did you take in a week?

Number of portions ____

If Yes, previously on 33.3:

DAILY USE OF SNUFF

33.3.4 If you used snuff daily previously, how many years since you stopped?

Number of years__

OCCASIONAL SNUFF USE

33.4 Do you use, or have you used snuff sometimes, but not daily?

Never Yes, now Yes, previously

If Never, skip to 34.1

If Yes, now or Yes, previously:

OCCASIONAL SNUFF USE

33.4.1 How many portions did you/do you usually take in a week?

Number of portions____

3 NOISE

How sensitive are you to noise? Tick next to the statement that best fits

34.1 I am sensitive to noise

Agree strongly

Quite agree

Agree slightly

Disagree slightly

Quite disagree

Disagree strongly

34.2 Do you have a hearing loss (one/both ears)?

No Yes

34.3 During the last 12 months, did you experience ringing in your ears lasting more than 5 minutes?

No Yes

If No skip to 34.4:

If Yes:

RINGING IN THE EARS

34.3.1 How frequently do you have ringing in your ears?

(Tick once)

Less frequently than every week

Each week, but not every day

Each day, but not all the time

Most of the time

34.3.2 How long do the periods with ringing usually last?

(Tick once)

A few minutes

10 minutes- 1 hour

Longer than 1 hour

34.3.3 Some people do not care about the sound, for others it feels very troublesome to have ringing in their ears. Please, indicate how bothered you are by the ringing in your ears. (0 Not bothered, 10 Worst imaginable pain)

0 Not bothered 10 Worst imaginable pain

Over the past 12 months or so, when you are at home, how much are you bothered, disturbed, or annoyed by the following sources of noise?

(Tick once for each line)

Not at all Slightly Moderately Very Extremely

34.4 Road traffic
34.5 Helicopter
34.6 Aircraft
34.7 Boat/ship/port
34.8 Building and construction work
34.9 Industrial and business activity
34.10 Neighbour/others outside your home
34.11 Others inside your home (e.g. who plays loud music, snoring partner, child who wakes up screaming at night etc.)
34.12 Other noise source

34.13 How many years in total have you worked in noisy locations where it has been necessary to shout to be heard?

Number of years___

If not Work full-time or Work part-time on 4.1, skip to 35.1

If Work full-time or Work part-time on 4.1:

34.13.1 Over the past 12 months, how much have you been bothered (by music, people talking, ventilation plant, machinery, equipment etc.) that has been disturbing your work?

Not at all

Slightly

Moderately

Very

Extremely

35 ORAL HEALTH

Next are questions concerning how your teeth and general dental health affect you in everyday life. First you will find questions regarding bothers with your teeth or dentures.

BOTHERS WITH TEETH/DENTURES

Every or nearly every day Once or twice a week Once or twice a month Less than once a month
Never affected

During the past 6 months:

35.1 During the past 6 months, how often have problems with your mouth and teeth caused you any difficulty with eating and enjoying food?

35.2 During the past 6 months, how often have problems with your mouth and teeth caused you any difficulty with speaking and pronouncing clearly?

35.3 During the past 6 months, how often have problems with your mouth and teeth caused you any difficulty with cleaning your teeth?

35.4 During the past 6 months, how often have problems with your mouth and teeth caused you any difficulty with sleeping and relaxing?

35.5 During the past 6 months, how often would you say that these ailments have made it difficult smiling and showing teeth without embarrassment?

35.6 During the past 6 months, how often would you say that these ailments have made it difficult maintaining usual emotional state?

35.7 During the past 6 months, how often would you say that these ailments have made it difficult enjoying contact with other people?

35.8 During the past 6 months, how often would you say that these ailments have made it difficult carrying out major work and a social role?

35.9 How often do you usually brush your teeth?

Once a week or less often

A couple of times every week

Once a day

Twice a day or more often

35.10 Do you go regularly to the dentist / dental care?

Yes, at least once a year

Yes, every year

Yes, every second year

Yes, with longer intervals than 2 years

No, only for acute problems

No, never goes

Do you use some of these oral hygiene products- and if so how often?

	Never/rarely	A couple of times every month	A couple of times every week	Daily

35.11 Fluoride toothpaste
35.12 Floss, interdental brushes and/or tooth-sticks
35.13 Fluoride tablets
35.14 Fluoride mouth rinse (Flux,Fluorid mouth rinse, Nycodent)
35.15 Antibacterial mouth rinse (Listerine, Corsodyl, Klorhexidin)?

Next are questions on how you experience a visit to the dentist. To what degree would you feel anxious in connection with a visit to the dentist?

Not anxious Slightly anxious Fairly anxious Very anxious Extremely anxious

35.16 If you went to your dentist for treatment tomorrow, how would you feel?
35.17 If you were sitting in the waiting room (waiting for treatment), how would you feel?
35.18 If you were about to have a tooth drilled, how would you feel?
35.19 If you were about to have your teeth scaled and polished, how would you feel?
35.20 If you were about to have a local anaesthetic injection in your gum, above an upper back tooth, how would you feel
?

36 SKIN AND DERMATOLOGY

Do you have, or have you had any of the following skin and dermatology diseases?

No Yes

36.1 Psoriasis
36.2 Psoriasis arthritis
36.3 Atopic eczema (children's eczema, atopic dermatitis)
36.4 Recurring hand eczema
36.5 Recurring large, painfull, lumps or abscesses which often heal with a scar in your armpits, groins or under your breasts (disease called hidradenitis suppurativa)

If Yes on 36.1:

PSORIASIS
36.1.1 Have you ever been diagnosed with psoriasis by a doctor?
No Yes

36.1.2 Have you had a psoriasis rash within the last 12 months?
No Yes

If Yes on 36.2:

36.1.2.1 Which description best describes/ described your psoriasis rash over the last 12 months?
(Tick once)

Sudden outbreak of tiny spots (less than one cm) spread over the entire body

Patches on the elbows/ knees/ scalp that appear sometimes

Patches on the elbows/ knees/ scalp that are almost always there

Patches on the elbows/ knees/ scalp that are almost always there, but also some patches on the torso/ upper body

Outbreaks of psoriasis rash on larger areas of the body occasionally

Psoriasis rash on larger area of the body, which are almost always present

If Yes on 36.2:

Psoriasis arthritis

36.2. Have you had symptoms of psoriasis arthritis within the last 12 months?

No Yes

If Yes on 36.3:

Atopic eczema (children's eczema, atopic dermatitis)

36.3.1 Have you had atopic eczema (atopic dermatitis) rash within the last 12 months?

No Yes

If Yes on 36.4:

Recurring hand eczema

36.4.1 Have you had a hand eczema rash within the last 12 months?

No Yes

If Yes on 36.5:

PAINFULL LUMPS (HIDRADRENITIS)

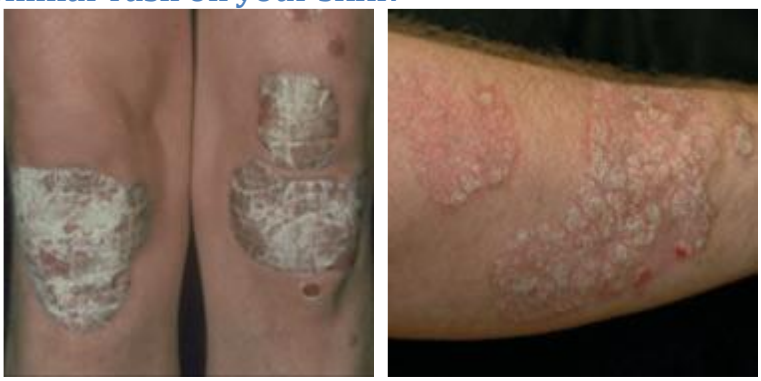
36.5.1 Have you had outbreaks/ symptoms of hidradenitis within the last 12 months?

No Yes

Do you have, or have you had:

(Tick once or more)

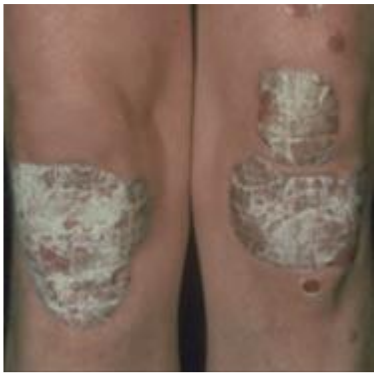
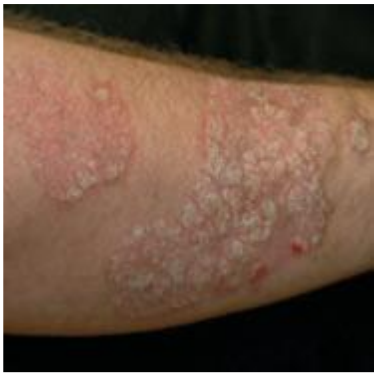
36.6 Similar rash on your skin?



No Yes

If No, skip to 36.7.

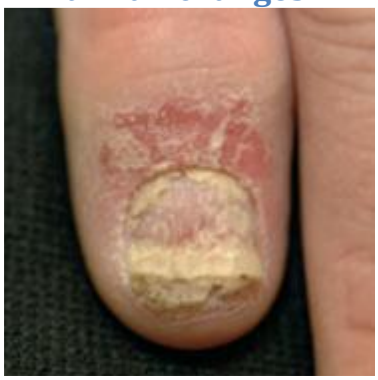
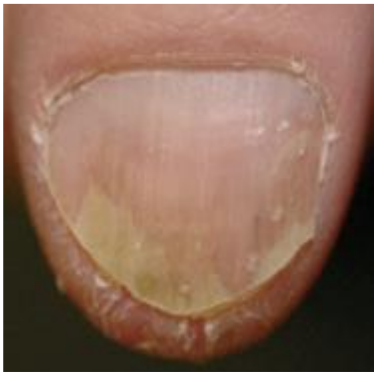
If Yes:

36.6.1 Have you had a similar rash within the last 12 months?

No Yes

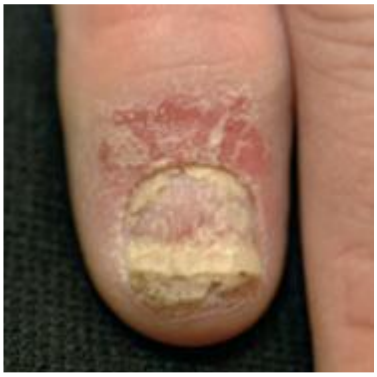
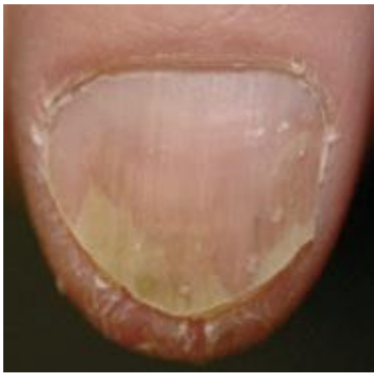
36.7 Similar nail changes?

No Yes

If No, skip to 36.8.

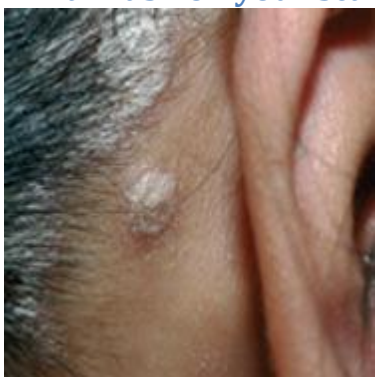
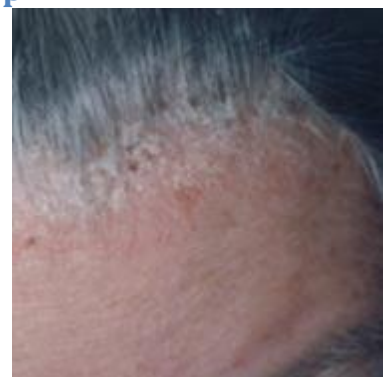
If Yes:

36.7.1 Have you had similar nail changes within the last 12 months?

No Yes

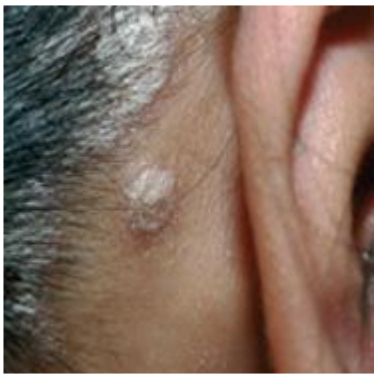
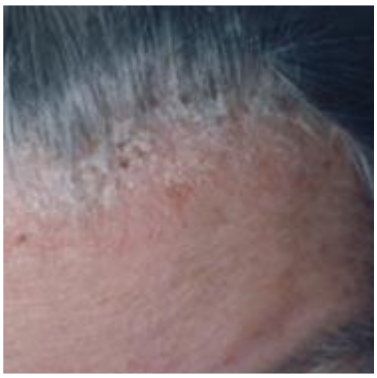
36.8 Similar rash on your scalp?

No Yes

If No, skip to 36.9.

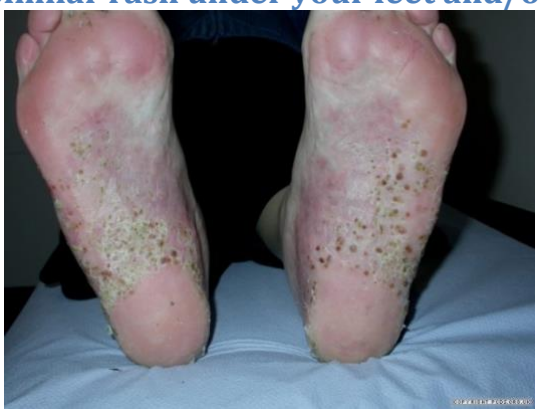
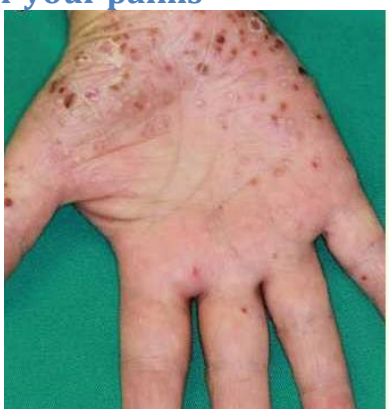
If Yes:

36.8.1 Have you had a similar rash within the last 12 months?

No Yes

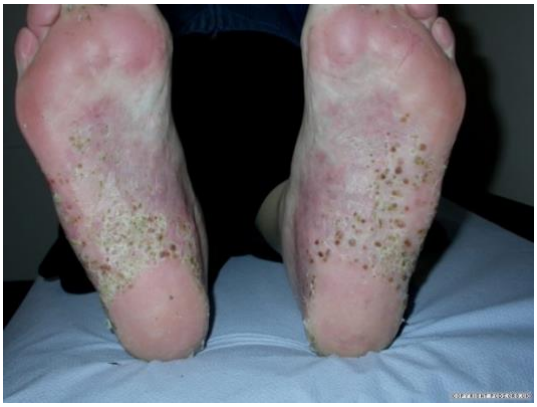
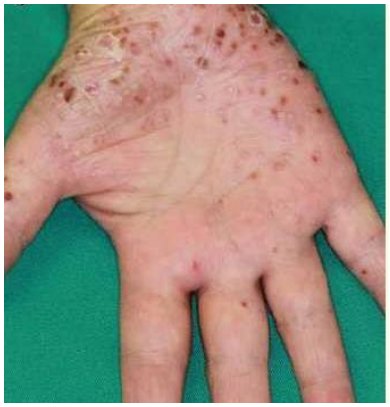
36.9 Similar rash under your feet and/or your palms

No Yes

If No, skip to 36.10.

If Yes:

36.9.1 Have you had a similar rash within the last 12 months?

No Yes

36.10 Have you had a rash or redness/irritation in your groin area, between your buttocks or under your arms which have lasted for more than 2 weeks at a time?

No Yes

37 TRAVEL AND ILLNESS

37.1 Please specify the number of travels you have performed during the past 12 months outside the Nordic countries with a duration of >1 week.

(Put in 0 if you do not have any travels of >1 week outside the Nordic countries in the past 12 months)

Number of travels in dropdown menu (1-20 travels)

If Travels=0 skip to 38.1

If Travels >0:

For each travel outside the Nordic countries with a duration of >1 week, tick the country you visited the longest, if you had diarrhoea during your stay and if you had a hospital admission and how many times:

For reise 1-20:

37.1.1 Countries

Dropdown menu countries (200 countries)

37.1.2 Diarrhoea

No Yes

37.1.3 Number of hospital admissions

(Put in 0 if you were not admitted to a hospital in connection with your travel)

Number of times__

If number of times hospital admission =0, skip to 38.1.

If number of times hospital admission >0:

For each hospital admission in a country outside the Nordic countries during the past 12 months please specify in which country, what month and for how long you were hospitalized

37.1.3.1 Country

Dropdown menu countries alphabetical (200 countries)

37.1.3.2 Month hospitalized

(e.g March 2015)

37.1.3.3 Duration of hospitalization

Number of days__

38 ANTIBIOTICS BOUGHT ABROAD

38.1 Have you bougth antibiotics abroad during the last 12 months (penicillin-like medicine to treat infection)?

No Yes

If No, questioner completed

If Yes:

38.1.1 How did you acquire it?

Prescription from doctor/dentist

From a pharmacy, without prescription?

Appendix 3.

In Paper I-II, the sleep outcomes were assessed a self-reported sleep questionnaire.
In contrast, the majority of previous intervention studies have applied the Pittsburgh Sleep Quality Index (PSQI). Differences and similarities between the two questionnaires are highlighted below:

	PSQI	**Study sleep questionnaire**
Similarities	5A: Cannot get to sleep within 30 minutes	Sleep onset latency
	5B: Wake up in the middle of the night or early morning	Sleep maintenance difficulties and/or early morning awakening
	5D: Cannot breathe comfortably and 5E: Cough of snore loudly	Information collected, but not applied as there were no differences between groups.
	7: During the past month, how often have you had trouble staying awake	Sleepiness/tiredness
	5F-G: Feeling too hot/cold 5I: Have pain	Information not collected in Paper II, but included as confounders in Paper I
Differences	Collects information on habitual sleep during the last month	Collects information on habitual sleep, and requires a duration of three months for insomnia diagnosis
	9: During the past month, how would you rate your sleep quality overall?	Dissatisfaction with sleep (not required for insomnia diagnosis)