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Vitamin D, Depression and Headache

- Results from the Tromsø Study and from an intervention study with vitamin D

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Contents

Acknowledgements

The work for this thesis was carried out at the Tromsø Endocrine Research Group, Department of Clinical Medicine, University of Tromsø and at the University Hospital of North Norway (UNN). I was halfway through my qualification as specialist in internal medicine, when I was presented with an opportunity to sign up as PhD-student and join the Endocrine Research Group. This was an excellent occasion to join an active research environment and learn how to do research from a clinical point of view. The D/Dep study had already started, and I began work on this thesis in October 2010 and finished in December 2012.

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Norwegian summary/Norsk populærvitenskapelig sammendrag

I Tromsøundersøkelsen 2007-08 deltok mer enn 12 000 personer. Alle fikk tatt blodprøver og svarte på spørreskjema om helsetilstanden. Vi har sammenlignet de som hadde høyt nivå av vitamin D med de som hadde lavt nivå, og vi fant at de med høyt nivå hadde 41% mindre risiko for å være deprimerte enn de med lavt. For å undersøke dette videre innkalte vi 257 personer til å delta i en studie hvor en skulle se om vitamin D tilskudd hadde effekt på depresjon. Halvparten fikk vitamin D og den andre halvpart placebo (narremedisin). Etter 6 måneders behandling var det ingen forskjell på de to gruppene, og således ingen gunstig effekt av vitamin D. Vi brukte flere forskjellige spørreskjema til å vurdere depressive symptomer, og våre analyser viste at disse skjemaene også er velegnet til å bruke i en frisk befolkning.

Videre fant vi i Tromsøundersøkelsen at det er en sammenheng mellom lavt nivå av vitamin D og hodepine. Personer med lavt nivå hadde 20% økt forekomst av hodepine i forhold til de med høyt. Det var ikke sammenheng mellom migrene og vitamin D.

List of papers

The thesis is based on the following papers:

Paper I. Kjærgaard, M., R. Joakimsen, and R. Jorde, *Low serum 25-hydroxyvitamin D levels are associated with depression in an adult Norwegian population.* Psychiatry Res, 2011. **190**(2-3): p. 221-5

Paper II. Kjærgaard, M., K. Waterloo, C.E. Wang, B. Almås, Y. Figenschau, M.S. Hutchinson, J. Svartberg, and R. Jorde, *Effect of vitamin D supplement on depression scores in people with low levels of serum 25-hydroxyvitamin D: nested case-control study and randomised clinical trial*. Br J Psychiatry, 2012. **201**(5): p. 360-8.

Paper III. Kjærgaard M, C.E.A. Wang, K. Waterloo, and R. Jorde, *A study of the psychometric properties of the Beck Depression Inventory-II, the Montgomery and Åsberg Depression Rating Scale and the Hospital Anxiety and Depression Scale in a healthy population*. Submitted for publication September 2012.

Paper IV. Kjærgaard M, A.E. Eggen, E.B. Mathiesen, and R. Jorde, *Association Between Headache and Serum 25-Hydroxyvitamin D; the Tromsø Study: Tromsø 6.* Headache, 2012. **52**(10): p. 1499-1505

Abbreviations

AUC	Area Under the Curve
BDI-II	Beck Depression Inventory-II
BMI	Body Mass Index
CES-D	Center for Epidemiologic Studies Depression Scale
CRH	Cortisol Releasing Hormone
CV	Coefficient of Variation
DBP	Vitamin D Binding Protein
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders-IV
GFR	Glomerular Filtration Rate
GSS	Global Seasonality Score
HADS	Hospital Anxiety and Depression Scale
HbA_1c	Glycosylated haemoglobin
ICD-10	International Statistical Classification of Diseases and Related Health Problems 10th Revision
ICHD	International Classification of Headache Disorders
IHS	International Headache Society
MADRS	Montgomery-Åsberg Depression Rating Scale
MDE	Major Depressive Episode
OR	Odds Ratio
PHQ-9	Patient Health Questionnaire-9
PTH	Parathyroid Hormone
RCT	Randomized Controlled Trial
ROC	Receiver Operating Characteristics
SAD	Seasonal Affective Disorder

SCID-CV	Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders-IV Axis I Disorders-Clinician Version
SCL-10	Hopkins Symptom Check List-10
SPAQ	Seasonal Pattern Assessment Questionnaire
TTH	Tension Type Headache
VDR	Vitamin D Receptor
WHO	World Health Organization
$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D

Introduction

Vitamin D was discovered in 1922 in the process of exploring the possible causes for rickets in children. Firstly, vitamin D was believed to be a true vitamin but later discovered also to be a hormone that could be synthesized in the human body when exposed to sunlight [1]. For decades the primary focus for vitamin D research was directed at the calcium and bone metabolism and its related diseases, but with the discovery of the vitamin D receptor (VDR) in practically all human tissues, new theories for disease mechanisms started to form [2]. Depression has been reported to be more frequent during winter-time where vitamin D levels are low [3], and in combination with the finding of the VDR in the brain, this has led to theories about an association between vitamin D deficiency and depressive symptoms. The research published so far has reported diverging results on this subject, and it has not been possible to establish whether vitamin D deficiency is a cause for depression or if it is marker of unhealthy lifestyle in persons with depression.

The main object of this thesis was to investigate if the association between vitamin D and depression is also found in a population living in the far North (69^{o}N), and further, by use of a randomized controlled trial (RCT) (the D/Dep study), to examine whether vitamin D supplementation has any effect on symptoms of depression in persons with low vitamin D levels. Since measuring depressive symptoms can be strongly dependent on the methods used [4], an additional validation study on the depression scales used by us in the RCT has been included in this work as well.

Further, the association between musculoskeletal pain and vitamin D [5], and higher frequency of headache at northern latitudes [6], has led to speculations about a possible association between headache and vitamin D deficiency. However, no population studies about headache, in which serum 25-hydroxyvitamin D (25(OH)D) has been measured, have been published. We had the opportunity to examine the data from the sixth survey of the Tromsø Study to see if any such association was found, and has included the results in this thesis.

Vitamin D

Vitamin D is one of the fat soluble vitamins and is essential for humans. It can either be produced in the skin by sun exposure or be obtained by the diet. It has two forms; ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3), which differs by the structure of a side-chain [7]. Vitamin D_2 is present in mushrooms and yeast products, but only rarely used in supplements in Norway. Vitamin D_3 is widespread in animal foods such as cod liver, fatty fish, egg yolk, and fortified dairy products [2] as well as supplements.

A normal varied Norwegian diet, including supplements, does seldom contain enough vitamin D [8] to meet demands of the recommended 400 IU/day suggested by the Nordic Nutrition Recommendation working group [9]. Production of vitamin D in the skin, catalyzed by sunlight, is therefore of great importance. The pro-vitamin D_3 (7-dehydrocholesterol) is derived from cholesterol and is abundant in the skin, and when exposed to UVB light with wavelength 290-320 nm it is converted by photolysis to pre-vitamin D_3 [10]. Pre-vitamin D_3 is unstable at body temperature and spontaneously transforms to vitamin D_3. Vitamin D intoxication does not happen due to extreme sun exposure alone, since the process is auto-regulated in the skin when exposed to excessive sunlight. Both pre-vitamin D_3 and vitamin D_3 can transform into biologically inert substances (tachysterol, lumisterol, suprasteroles) by further exposure to UVB light, and only about 15% of the available 7-dehydrocholesterol is transformed to vitamin D_3 under optimal light conditions [10, 11]. The production varies with skin pigmentation, age, use of sunscreen, cultural factors that affect clothing and sun seeking habits, and with time of day and season. In Tromsø (69^oN) sufficient light for photolysis is present from mid-March till the end of September in optimal weather conditions, but with cloudy conditions the season is much shorter [12]. Furthermore, genetic variations in cholesterol metabolism have been shown to affect the production of vitamin D_3 by limiting the amount of available 7-dehydrocholesterol [13].

Vitamin D absorbed from the gut or produced in the skin is inactive and is transported to the liver attached to the vitamin D Binding Protein (DBP), where it is hydroxylated to either $25(OH)D_2$ or $25(OH)D_3$ by 25-hydroxylase [14]. For simplicity, the two metabolites will be referred to as 25(OH)D in the following, unless distinction is important. 25(OH)D is further hydroxylated to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) by 1α-hydroxylase. This process is part of the calcium metabolism and mainly happens in the kidneys, but 1α-hydroxylase has also been located in many other tissues suggesting additional effects of vitamin D [15]. The 1α-hydroxylase activity in the

kidneys is up-regulated by parathyroid hormone (PTH), which is regulated through a negative feedback mechanism in the parathyroid glands dependent on the calcium level in serum. $1,25(OH)_2D$ leads to increased calcium absorption in the gut, reduced excretion in the kidneys and increased calcium resorption from bone, which result in increased serum calcium. High serum calcium leads to production of fibroblast growth factor 23 from bone cells, which down-regulates 1α-hydroxylase and thereby reducing serum calcium [15]. The 1α-hydroxylase located in other tissues is not regulated by PTH, but probably by local cytokines and growth factors and thus, $1,25(OH)_2D$ production is dependent on amount of available 25(OH)D. The amounts produced are supposedly small and are believed to function in an autocrine and paracrine manner [15].

$1,25(OH)_2D$ is a lipophilic hormone and is transported to its target tissues attached to DBP, which is a transport protein with specific properties for binding of vitamin D metabolites. Only a very small fraction of the vitamin D metabolites are circulating as free steroids with free $1,25(OH)_2D$ estimated to be less than 1% of the total. DBP's affinity for the different vitamin D metabolites varies, being high for 25(OH)D and lower for $1,25(OH)_2D$. Whereas the VDR in the target tissues has the opposite properties, with highest affinity for $1,25(OH)_2D$, and thus resulting in low amounts of unbound vitamin D metabolites [15].

The effects of $1,25(OH)_2D$ are mediated through regulation of transcription of genes in the target cells, which is facilitated by the VDR, a ligand-activated transcription system [15]. The majority of genes regulated are related to calcium metabolism, but growing evidence supports that it also affects inflammation and cell differentiation [16]. Furthermore, $1,25(OH)_2D$ has non-genomic effects, but the regulation and mechanisms of these are not fully understood [15]. This multitude of possible effects of vitamin D suggests that vitamin D is more than a regulator of calcium metabolism, and vitamin D has so far been associated with cancer [17], diabetes [18], autoimmune diseases [19], infectious diseases [20], cardiovascular disease [21] and overall mortality [22].

The half-life of 25(OH)D is two-three weeks, and that of $1,25(OH)_2D$ is 2-4 hours [15]. The primary way of deactivation is by 24-hydroxylation by vitamin D 24-hydroxylase, which is present in the target cells. The expression of 24-hydroxylase is part of the calcium regulation system and is increased with low PTH levels. The end products are eliminated via the kidneys or liver (bile) [15].

Though 25(OH)D is also stored in fat tissue [2], serum 25(OH)D is currently the most accurate indicator of vitamin D status due to its relatively long half-life [15]. There is no consensus on what is "normal range" for serum 25(OH)D, and traditionally recommendations have been based

on what levels are sufficient to avoid rickets. Thus, serum 25(OH)D < 25 nmol/l is considered deficiency, levels from 25 to 50 nmol/l suboptimal, and > 50 nmol/l to be a sufficient level of serum 25(OH)D to avoid disease [23]. Different models have been proposed to investigate which 25(OH)D level is "natural" and optimal for health, with some suggesting that people living near equator and wearing little clothing are bound to have the most "natural" level, which is found to be around 115 nmol/l [24, 25]. Others suggest using the serum 25(OH)D level of which PTH stops to decrease as an indicator of a healthy vitamin D level; the results have been diverging, but a cut-off at 75nmol/l has been suggested [26]. Another approach is not to find what is supposedly natural, but to find which level results in the best health. Several population studies with hard end-points such as mortality, cardiovascular mortality and cancer risk indicated that 25(OH)D level > 100 nmol/l is beneficial [27, 28], and this has led to suggestions about revising the recommendations with a 25(OH)D level > 75nmol/l as cut-off for sufficient status [29]. But few RCTs with hard end-points have been published, and population studies do not yield sufficient data to conclude about causality, which will be discussed in detail below. So far the recommendations in Scandinavia are unchanged with regard to recommended serum 25(OH)D level [9].

Depression

Depression is a worldwide problem causing great morbidity for the individual and socio-economic costs for the society. Currently, it is reported by the World Health Organization (WHO) to be the fourth leading cause for disease burden, and is estimated to be the second leading cause by 2030, second only to HIV/AIDS [30]. The reason for this impact is partly related to prevalence, but early age onset, risk for discontinuation of education, and long periods of reduced work capacity, play important roles as well [31, 32]. Reports on prevalence vary between populations and can be difficult to compare due to different definitions and methods used. A recent Norwegian population study reported a point prevalence of 9.7% based on self-compiled questionnaires [33], whereas a WHO report, based on diagnostic interviews, found results ranging from 2-4% [34]. Life-time prevalence is even more difficult to investigate, but a world average is found to be in the range 8-12% [32].

Depression, or major depressive disorder, is defined in the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) from the American Psychiatric Association as having had at least one major depressive episode (MDE) of which the criteria are listed in Table 1. The MDE must not be related to a schizoaffective disorder or other conditions with schizophrenia, and no

episodes with mania or hypomania can ever have been present. Major depressive disorder can be categorized according to recurrence into either a single episode or recurrent episodes. To be defined as recurrent, there must be an interval of two months between two episodes where the person does not fill the criteria for MDE [35]. The ICD-10 criteria differ slightly from the DSM-IV criteria, but this is of minor importance to the present work and will not be elaborated further.

Table 1. DSM-IV Criteria for Major Depressive Episode

A. At least five of the following symptoms present during a two week episode, **and** presence of either depressed mood (1) or/and loss of interest (2)
1. Depressed mood
2. Loss of interest/pleasure
3. Unintentional weight loss or gain
4. Insomnia or hypersomnia
5. Psychomotor agitation or retardation
6. Fatigue/loss of energy
7. Feeling of worthlessness or guilt
8. Diminished ability to concentrate, think or make decisions
9. Thoughts of death or suicide
B. Symptoms do not meet the criteria for mixed episode (Bipolar disorder)
C. Symptoms cause distress or impairment
D. Symptoms are not caused by substance abuse, medication or general medical condition
E. Symptoms not better accounted for by bereavement or other conditions
A-E must be met to fill the criteria for MDE

Table adapted after DSM-IV [35]

Depression can further be divided into several subtypes of which Seasonal Affective Disorder (SAD) is the most relevant for this thesis. It is defined as a specifier to depression in both DSM-IV and ICD-10 and not as a specific disorder. According to DSM-IV there has to be a pattern of recurrent depression with at least two-thirds of the episodes following a seasonal pattern [35]. Though it is believed to be rather common, with 5-15% reporting some kind of seasonal variation in depressed mood [36], only about 1% of the population were found to fill the criteria in an American population study [37]. Furthermore, some authors have raised doubt about the plausibility of SAD as a disorder [38], since the association with latitude is at best weak, and since depression is not more common during the dark months in Northern Norway than in summer-time [39].

The pathophysiology of depression is complex and only partly understood. Evidence of hereditability has been found, especially in severe and recurrent depression [40, 41], but the risk of depression may in addition be influenced by personality traits, which are also hereditary, and a clear distinction can be difficult to make [41]. So far only a few genes which influence the risk of depression have been identified [42], and they seem to play only a small part in total risk.

Different theories about disease mechanisms of depression exits, the two best understood and explored being the mono-amine deficiency theory and the stress theory. In the mono-amine theory depression is thought to be caused by deficiency of mono-amines (serotonin, norepinephrine) in the brain [43]. Several processes, which lead to reduced level of these neurotransmitters and their second-messengers, both presynaptic and postsynaptic, have been identified in depressed persons and in post-mortem autopsies. These processes span from reduced production of neurotransmitters, reduced release of transmitters, and reduced neurotransmitter receptor sensitivity to altered gene expression [43]. That anti-depressants, which increase the supply of mono-amines are effective support this theory, but that only two-thirds of patients respond to this treatment suggest that other mechanisms are involved as well.

The foundation for the stress theory is based on the observation that depression is often seen in individuals who have experienced stressful situations. The stress hormone cortisol is up-regulated by corticotropin-releasing hormone (CRH) via the hypothalamic-pituitary-adrenal axis. CRH is produced in the hypothalamus and regulated by negative feedback from cortisol. In depressed individuals this negative feedback mechanism is thought to be disturbed, leading to both elevated cortisol and CRH levels [44, 45]. Why this leads to depression is uncertain, but some suggest that reduced neuroregeneration in the hippocampus may play a role [43]. So far no single theory has

produced sufficient explanation for the mechanisms of depression, and it is generally believed that the pathophysiology of depression is multifactorial with several mechanisms involved [4].

Measuring depression

To diagnose depression correctly according to the DSM-IV criteria an objective evaluation of psychomotor pathology is obligatory. A personal interview can be a time consuming process, and therefore several diagnostic instruments have been developed to make the process more effective. One review report that more than 280 measures of depression severity have been developed since 1918 [46]. Although recent studies suggest that a one-question approach (Are you feeling sad/depressed?) has as high sensitivity and specificity as acknowledged questionnaires [47, 48], most instruments still take form as a regular questionnaire. Also semi-structured interviews are used.

The purpose of the instruments differ, some are designed for screening (Hospital Anxiety and Depression Scale (HADS)) [49], some for measuring severity of depression in patients (Beck Depression Inventory (BDI-II)) [50], and yet others have been designed for measuring change in depressive symptoms in clinical trials (Montgomery-Åsberg Depression Rating Scale (MADRS)) [51]. Furthermore, they may contain different dimensions of depression and be specifically designed for a sub-population of patients. These aspects must be taken into consideration when using this type of instruments to study depression, especially when comparing results from different instruments and using them to set a diagnosis. In addition, it is of importance that the instruments are validated for the population in which they are used. In validation of instruments, some factors of importance are; internal consistency (how well each item/question measure the same construct i.e. depressive symptoms), specificity and sensitivity, concurrent validity (how well different instruments correlate with each other), and inter-rater agreement (how accurate the instrument are in setting a diagnose compared with the gold standard) [52]. The instruments used in our studies will be described in detail in the Methods section.

Vitamin D and depression

There is growing evidence that vitamin D plays a role in human brain development and function, though the details of the mechanisms remain unclear. 25(OH)D and $1,25(OH)_2D$ have both been found in the cerebrospinal fluid [53] and have been shown to cross the blood-brain barrier

[54]. Furthermore, active vitamin D metabolism and catabolism in the brain has also been established with the finding of 1α-hydroxylase and the VDR in substantial areas of the human brain [55]. The 24-hydroxylase has been found in rat brains that seem to be very similar to human brains with regard to vitamin D, but no conclusive evidence about its presence in human brains have been reported so far [56]. With regard to depression, the VDR and 1α-hydroxylase are also found in the hippocampus [55], an area which is found to be reduced in volume in persons with depression [57]. The effect of vitamin D is this area is unclear, but one *in vitro* study reported an neuroprotective effect of $1,25(OH)_2D$ in hippocampal cells when exposed to glucocorticoids [58], implying that vitamin D could have favorable effects by protecting against stress induced depression. Another possible effect of vitamin D in depression pathophysiology, is that vitamin D has been shown to enhance the transcription of genes encoding for tyrosine hydroxylase, which is involved in the regulation of norepinephrine production [59]. This effect have so far only been found in adrenal glands and not the brain, and further studies are needed to confirm if a similar mechanism is found in brain tissue. Animal studies on depression are limited; one study on mice without functioning VDR showed increased anxiety, but other depressive traits such as anhedonia (inability to experience pleasure) was not present [60], whereas a similar study on rats did not find neither anxiety nor depressive signs [61].

Numerous large population studies of the association between vitamin D and depression have been published. The results were at first diverging with some studies reporting an association [62-65] whereas others did not find such an association [66, 67]. However, in the recent years the evidence is growing with a number of studies reporting an inverse association between vitamin D and depression [68-72]. Only one recent study did not report a significant association, but it was a small study with only 118 participants [73], and the authors did report a trend towards an association. This development might be a result of publication bias, but different populations studied and methods used might also account for the inconsistency. Especially the methods used to diagnose depression varied, some used Center for Epidemiologic Studies Depression Scale (CES-D) [62, 65, 67, 71], BDI-II [63], Patient Health Questionnaire PHQ-9 [66], and some used less known instruments [64, 68]. Furthermore, adjustment for confounders varied, some did not include physical exercise [64], marital status [62] and educational level [63], whereas others included altitude [68], dietary intake [70] and urbanization level [64]. Though the association seems to be well documented, the question about causality remains unanswered from these studies.

Few RCTs have been reported to date, and the majority has been in non-clinical (not depressed) populations, and the results diverge. One small study by *Vieth et al*. reported positive effect on "well-being" of 4000 IU vitamin D/day compared to 600 IU/day after 6 months of treatment in persons with low serum 25(OH)D, but when the study was repeated 12 months later in persons with even lower serum 25(OH)D the effect was not significant [74]. Furthermore, *Sanders et al*. [75] did not find any effect on mental well-being of 500 000 IU vitamin D administered once yearly for up to five years in 2258 elderly women. On the other hand, *Lansdowne et al.* [76] found a positive effect on "mood" in 44 persons of 800 IU or 400 IU vitamin D taken together with vitamin A for five days compared with vitamin A alone, and *Kajehei et al.* [77] reported a beneficial effect of nine days treatment with 200 IU vitamin D and 500 mg calcium on general health scores related to depression in 60 women treated during menstrual cycles. These studies all used self-constructed well-being questionnaires or part of general health questionnaires, none of which are validated to assess for depression. In addition, the short time frame and small doses used in the last two studies might indicate that other factors were involved as well.

Only three studies in non-clinical populations have used validated depression scores to monitor the effect of vitamin D, and two of these used the BDI questionnaire. *Jorde et al.* [78] found a significant positive effect of 40 000 and 20 000 IU vitamin D/week for one year compared to placebo in 334 obese persons. On the contrary, *Dean et al.* [79] found no effect on BDI score in 63 healthy young persons after six weeks of treatment with 5000 IU vitamin D/day compared to placebo. The third study by *Bertone-Johnson et al.* [80], which by far is also the largest, used the validated Burnam score (a combination of CES-D and the Diagnostic Interview Schedule) in combination with information about use of anti-depressants to assess for depression. More than 36 000 women in the Women's Health Initiative Calcium and Vitamin D Trial were randomized to either placebo or 400 IU vitamin D + 1g calcium daily and followed for three years (mean). The authors reported no effect of vitamin D on depression compared with placebo after two years follow-up, but some methodological concerns must be noted. Firstly, the participants were allowed to continue with the vitamin D supplements they used at inclusion with an upper limit of 600 IU/day, which was later changed to 1000 IU/day. This might have masked an effect of a relatively modest 400 IU dose. Secondly, the majority of the participants were only evaluated at follow-up for depression by the use of antidepressants and not Burnam scale. This might have led to considerable misclassification, since anti-depressants have alternative uses as well (chronic pain, fibromyalgia, panic disorder). On the other hand approximately 2300 participants did complete the Burnam

questionnaire, and no effect was found here either. Only one randomized study on clinically depressed patients has been published to date. *Khoraminya et al.* [81] recruited 40 out-patients with major depressive disorder to either treatment with Fluoxetine 20 mg or Fluoxetine 20 mg + 1500 IU vitamin D daily for eight weeks, and found that patients in the vitamin D group had significantly lower BDI and Hamilton Depression Rating Scale score (HDRS) from week four and onwards. The effect of vitamin D was rather strong (change in HDRS 11.7 vs. 17.2), and further well designed studies are needed to confirm this finding.

Headache

Headache is a worldwide problem; a recent review found a global 1-year prevalence of 47% of Tension-Type Headache (TTH) and migraine combined, and the authors estimated headache to be one of the 10 most disabling conditions overall [82]. In addition to individual morbidity it results in great costs for the society as well [82]. In Norway a study from year 2000 found a 1-year prevalence of overall headache of 38%, where 12% was classified as migraine and 26% as non-migraine headache based on slightly modified criteria of the International Classification of Headache Disorders (ICHD-II) definition [83, 84]. Migraine is more prevalent in women with a male:female ratio of 1:2 to 1:3, whereas TTH is more evenly distributed between men and women with a ratio of 4:5 [85]. It has been reported that both TTH and migraine are underdiagnosed and undertreated [86, 87], and recently a global initiative has been started to heighten awareness [88].

According to the ICHD-II [84] headache can be divided into primary and secondary headache based on assumed pathophysiology. Secondary headaches are caused by external factors such as medication overuse or head/neck trauma, whereas in primary headaches no specific structural disturbances can be found in the brain. TTH and migraine, which are investigated in the study included in this thesis, are categorized as primary headaches and described in detail in the following paragraphs.

Tension-Type Headache

TTH is has many clinical presentations, which can partly be explained by the wide diagnostic criteria suggested by ICHD. It is partly an exclusion diagnosis, since symptoms typical of migraine (nausea, vomiting, photophobia and phonophobia present at the same time, aggravation by physical activity) cannot be present, but several characteristics have to be present as well. These include

bilateral location, pressing (non-pulsating) quality, and mild to moderate intensity. Furthermore, headache of more than 30 minutes duration must have been present for at least 10 days the past year. It can further be divided into subgroups according to frequency (non-frequent episodic, frequent episodic, and chronic) and based on the presence or absence of pericranial tenderness [84].

TTH is found to be associated with stress and mental tension [89], sleeping patterns [89], some personality types and coping strategies [90, 91], depression [92], and low level of physical exercise [93]. The pathophysiology of TTH is not fully understood, but probably both peripheral and central factors are involved [94]. Although the peripheral factor pericranial tenderness, which is found in patients suffering from TTH [95], seems to be an important clinical issue, only limited evidence to support this theory has been found. Some studies suggest sensitizatation of peripheral nociceptors [96] and increased electromyographic activity in triggerpoints [97] to play a role, whereas other theories involving muscle ischemia [98] and general increased muscle tone [95] have not been supported. Growing evidence now support that central factors are of greater significance, especially sensitizatation of neurons at spinal and supraspinal levels [94] as well as decreased inhibition of nociceptive signals [99] leading to an increased sensitivity to pain and thereby causing headaches.

Migraine

Migraine is a common condition with a reported worldwide lifetime prevalence of 14% with onset early in life (second and third decade) and a peak prevalence in the fourth decade [85]. Aura, which is visual disturbances in the beginning of a migraine attack, was reported in 31% of migraineurs, 18% did experience aura with every attack, whereas 12% had both headache with and without aura [100]. Migraine has been reported to be associated with smoking, body mass index (BMI), low alcohol consumption and low physical activity in a recent Norwegian study [93]. Other studies describe associations with stress and mental tension [89], sleep patterns [101] and educational level [102]. The higher prevalence of migraine in women, and the attacks relation to the menstrual cycle, are found to be associated with hormonal influences [103], and a sub-type is accordingly named Menstrual Migraine in the ICHD [84]. The ICHD criteria for migraine are presented in Table 2.

Table 2. Diagnostic criteria for migraine according to ICHD.
A. At least five attacks fulfilling criteria B-D
B. Headache lasting 4-72 hours (untreated)
C. Headache has at least two of the following characteristics
Unilateral location
Pulsating quality
Moderate to severe pain intensity
Aggravation by physical activity
D. Presence of at least one of the following symptoms during headache
Nausea and/or vomiting
Photophobia and phonophobia
E. Not caused by other conditions
Migraine can further be divided into migraine without aura or migraine with aura Adapted after ICHD [84]

The understanding of mechanisms causing aura and headache is developing with new neuroimaging possibilities. Evidence now supports a neurovascular mechanism, in which it is suggested that a migraine attack is precipitated by hyperexcitability in the cortex [104] followed by a slowly spreading depression of neuronal activity in particular in the occipital region, which simultaneously leads to hyperoxygenation [105]. This is thought to be the mechanism of aura, but is also seen in persons without aura suggesting a linkage with headache [106]. The pain mechanism is poorly understood, but it has been suggested that activation of the trigeminal-vascular system by the spreading wave of neuronal depression may lead to vasodilation of intracerebral arteries followed by local extravasal inflammation causing pain [107]. In addition, disturbance of the serotonin metabolism is considered to be of importance [108].

Vitamin D and headache

The association between vitamin D and headache has only been studied to a very limited degree. A study by *Salvesen et al*. [109] found a higher incidence of TTH during winter time in Northern Norway supporting a vitamin D connection, but did also report an increased incidence of migraine attacks during summer-time. A recent Japanese study described a significant association between migraine and a gene coding for DBP, but the full study has not been published in English and details are not available [110]. Only one population study has been published, a Norwegian cross-sectional study reporting significantly lower levels of serum 25(OH)D in 63 patients with headache compared with patients with musculoskeletal pain [5]. The patients were selected from a GP's office based on an assumption of hypovitaminosis D, and even though adjustments were performed for age, gender, season and country of origin, the study design might induce considerable confounding and the results must be interpreted with caution. In support of an association, one meta-analysis by *Prakash et al.* [6] based on headache prevalence in different countries found a significant association between latitude and headache prevalence, suggesting that vitamin D might be of importance in headache pathology. However, since the studies included in the meta-analysis were pure prevalence studies there was no information about serum 25(OH)D available, and since serum 25(OH)D has been shown not to uniformly decrease with latitude [111], confounding by factors such as lifestyle, cultural differences, and age distribution might explain the results [112]. Likewise, two abstracts from the Annual Meeting of the American Headache Society reported low serum 25(OH)D levels in headache patients [113, 114], but the studies have not been published in full and details about adjustment for confounders were not reported.

No RCTs about the effect of vitamin D on headache have been published; only three case-reports are available. *Prakash et al.* [115] reported effect of vitamin D supplement in eight patients with TTH. These patients did however also suffer from osteomalacia and their headaches might be related to bone-pain. *Thys-Jacobs* [116, 117] has reported that four women with migraine, two with menstrual migraine and two with postmenopausal migraine, had beneficial effect of treatment with vitamin D and calcium supplements. Even though these studies might indicate an effect of vitamin D, they were small and uncontrolled, and the placebo effect must be considered.

A plausible suggestion for a mechanism of vitamin D in headache has not been published. However, since serotonin metabolism is involved in migraine pathophysiology [108], and vitamin D might affect serotonin production via tyrosine hydroxylase [59], a possible connection could be

hypothesized. Furthermore, vitamin D has been shown to down-regulate inflammatory parameters [16], and this might be important in regulating the extravasal inflammation related to pain in migraine [107]. Finally, a linkage between vitamin D and migraine could be hypothesized to be related to calcium channels, since susceptibility to migraine is suggested to be mediated by abnormal function in these channels [118], and vitamin D has been shown to affect the calcium channels in hippocampal cells [119]. Vitamin D might also be related to chronic musculoskeletal pain [120], and an association with the peripheral factors in TTH could be hypothesized. On the other hand, the evidence is not convincing [121] and other factors might be of greater importance.

Aims of the thesis

1) To examine the association between serum 25(OH)D and depressive symptoms

2) To examine whether vitamin D supplements has any effect on depressive symptoms

3) To examine the properties of the BDI-II, MADRS and HADS in measuring depression in a healthy population

4) To find optimal cut-off points for BDI-II, MADRS and HADS for screening for depression in a healthy population

5) To examine the association between vitamin D and headache

Study population and methods

The methods used are described in detail in each paper and the following paragraphs are meant to summarize in addition to adding supplementary information where appropriate.

The Tromsø Study

The Tromsø Study is a longitudinal population based study with the first survey performed in 1974. It was originally intended to investigate lifestyle factors related to cardiovascular disease in men and to shed some light over why the prevalence of these diseases was so high in Northern Norway. The following surveys have also included women and the number of participants has increased, with the highest number in 1994-95 where all inhabitants in Tromsø Municipality aged 25 years or older were invited (Tromsø 4, 27 158 participants). In the newer surveys, in addition to cardiovascular disease, other areas such as endocrinological, neurological, dermatological, gastrointestinal and psychological diseases have also been investigated, and a wide array of possible causes and confounders have been included [122].

For Papers I and IV data from the sixth survey of the Tromsø Study (Tromsø 6) have been used. It was carried out in 2007-2008 and a total of 12 984 persons participated. The participants were invited from two groups; one group included all the participants from Tromsø 4 who had taken part in a special follow-up study. Persons invited for this follow-up were all persons aged 55-74 years plus a random sample of persons in the age groups 25-54 and 75-85 years, a total of 7 965 participated. The other group that was invited for Tromsø 6 was a 10% random sample of all persons aged 30-39 years, a 40% random sample of all aged 43-59 years and everybody aged 40-42 years or 60-87 years. Overall a total of 19 742 were invited, resulting in a participation rate of 65.7% [122]. The participants were not asked about ethnicity, but due to the demographics of Tromsø practically all the participants were considered to be Caucasians.

The D/Dep study

In the D/Dep study, which are the fundament for Papers II and III, participants were recruited based on their serum 25(OH)D level in Tromsø 6. In addition to the intervention part, the study had a nested case-control design, where persons with low serum 25(OH)D levels were considered cases,

and those with high levels were controls. Eligible persons were aged 30-75 years and had either serum 25(OH)D below the 20 percentile (< 55 nmol/l) or above the 75 percentile (> 70 nmol/l). The persons with the most extreme serum 25(OH)D values were invited first to obtain the largest possible difference in serum 25(OH)D level between the cases and controls. Persons with known diabetes, active cancer or cancer diagnosed in the past five years, kidneys stones, stroke or coronary heart disease in the past 12 months, pregnant or lactating women, women below 50 years without adequate contraception, or persons reporting use of either vitamin D supplements, antidepressants or mood stabilizing medication, or planning a trip to a sunny location in the study period were excluded from the study. Furthermore, participants with possible primary hyperparathyroidism (PTH > 5.0 pmol/l combined with serum calcium > 2.50 mmol/l), men with serum creatinine >130 mmol/l and women with serum creatinine > 110 mmol/l, and participants with systolic blood pressure > 174 mmHg or diastolic blood pressure > 104 mmHg were not included in the intervention study. Persons with serious depression in the SCID interview, BDI-II score > 29 or MADRS score > 34 were offered a consultation with a psychiatrist and were excluded from the intervention study. A total of 1351 persons were found eligible for the study and invited, of the 472 who responded 357 were included in the case-control part of the study, and 243 were randomized for the intervention part of the study.

Questionnaires (Papers I and IV)

In Tromsø 6 participants filled in questionnaires, including questions about medical history, lifestyle and socio-economic factors. The first part of the main questionnaire were completed by the participants before they came to the first visit and then handed in at attendance. The second part was given to the participants immediately after the visit, with most completing it before leaving the clinic, and a smaller number completing it later and returning it by mail.

The following variables were constructed from the questionnaires (see appendix I for complete questionnaire): current smoker (yes/no) with former smoker assigned to the non-smoker category, marital status (living with spouse/partner (yes/no)), educational level (college or higher (yes/no)), and alcohol consumption units/week. A physical activity index was estimated in hours/week, where light activity was given half weight and hard activity double weight compared with moderate activity. In Paper I chronic disease was defined as having been admitted to a hospital or been to a consultation at a hospital in the past year (yes/no), thus using contact with a hospital as

a proxy for chronic disease. In Paper IV, total number of self-reported diseases diagnosed by a physician (hypertension, diabetes, asthma, stroke) was used as a measure for chronic disease.

Other measurements (Papers I, II, IV)

Weight was measured with the participants wearing light clothing and no shoes. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Blood pressure was measured after 10 minutes rest, three measurements were done and an average of the last two measurements was used in the analyses.

Laboratory analyses

Several methods for quantification of 25(OH)D in serum exist and so far there has not been agreed upon a common international calibrator [123], which makes the results from different methods a challenge to compare. Currently an international committee is working on finding a common standard, but at the moment of writing no consensus has been presented [124]. In the studies presented in this thesis, two different methods were used. In Papers I and IV serum $25(OH)D_3$ was measured by immunometry (electrochemiluminescent immunometric assay) using an automated clinical chemistry analyzer (ELICA; Modular E170; Roche Diagnostics GmbH, Mannheim, Germany). The producer states a total analytical precision (coefficient of variation (CV)) of ≤ 7.8% judged in any of three different concentrations (48.6–73.8–177.0 nmol/l). The analytical sensitivity was 10 nmol/l, and cross-reactivity with $25(OH)D_2$ was < 10%. A reference range of 27.7-107.0 nmol/l was suggested as guideline by the manufacturer. This analysis has been approved by Norwegian Accreditation, but has later been show to overestimate serum 25(OH)D in smokers [125], and has now been removed from the market.

It has earlier been show that serum 25(OH)D is stable even after several freeze-thaw cycles [126], and for the study in Paper II serum samples for 25(OH)D from baseline and 6 month were stored at -70 degrees Celsius and analyzed after the study was completed. Measurement was performed using an isotope dilution tandem mass spectrometry method (LC-MS/MS method) developed at the Hormone Laboratory, Haukeland University Hospital, Bergen, Norway. The within-day precision (CV) was ≤ 3.1%, and the between-day precision (CV) was ≤ 8.7%. There is no known interference from other substances, including no cross-reaction with vitamin $25(OH)D_2$ [125].

All other laboratory analyses were performed consecutively at the Department of Medical Biochemistry at the University Hospital of North Norway.

Depression measurements

Hopkins Symptom Checklist (SCL-10) (Paper I)

The Hopkins Symptom Checklist was developed in 1973 [127]. It originally consisted of 90 questions and was designed to measure the level of stress. It has later been revised and validated in shorter versions, especially the 25 and 10-item versions have been validated in measuring anxiety and depression in a general population [128, 129]. The SCL-10, used in Tromsø 6, is a self-compiled questionnaire consisting of 10 questions regarding how the participants have been feeling for the last week (see appendix 2). They give a score from 0 (not at all) to 4 (a lot/all the time) on each question and a total score is calculated by adding all the answers and dividing by 10. A score $\geq$ 1.85 is considered to be the cut-off for depression and/or significant mental distress [130]. The scale can further be divided into a sub-scale for anxiety using the first four questions and a sub-scale for depression using the last six, but these have only been validated to a limited degree. Missing values (1-2 questions) were imputed using the sample mean for each question, and persons with three or more missing values were excluded.

BDI-II (Papers II and III)

The Beck Depression Inventory originates from 1961 [131] and has been validated to measure depression level in both psychiatric and general populations [132]. A revised second edition (BDI-II) was published in 1996 to accommodate the changes in the DSM-IV classification, changing all but three questions and expanding the timeframe from one to two weeks [50]. It is a self-report questionnaire consisting of 21 questions (see appendix 3) with the participants giving each a score from 0 to 3 (0 being no symptoms, and 3 being severe symptoms). A total score is calculated by adding all the answers, thus giving a range from 0 to 63. The scale can further be divided into two sub-scores; a cognitive-affective using questions 1-13, and a somatic-vegetative using questions 14-21 [133]. The second edition has also been widely validated with regard to content and reliability in both psychiatric, somatic and general populations [50, 134-138], but although it is widely used as a screening instrument, only a limited number of studies have tested its ability as such [139]. The

most frequently used cut-offs values for screening are ≥ 14 for mild depression and ≥ 20 for moderate depression as originally suggested by Beck and colleagues, based on data from psychiatric out-patients [50].

HADS (Papers II and III)

The Hospital Anxiety and Depression Scale was designed in 1983 with the specific purpose to measure and screen for anxiety and depression in a somatic hospital population [49]. It is widely used and has been validated in both somatic, psychiatric and general populations [140]. It consists of 14 questions, 7 regarding anxiety and 7 regarding depression, to which the participants give an answer from 0 to 3, with 0 being the least depressed/anxious and 3 the most (see appendix 4). A total score is calculated by adding all the values. Furthermore, sub-scales of anxiety and depression can be calculated by using only the 7 questions for each category. A HADS-D of ≥ 8 seems to been generally agreed upon as to be the optimal cut-off to screen for depression [140, 141], whereas the literature on the optimal cut-off value for diagnosing depression with total HADS-score is more diverging. A cut-off of ≥12 is often used, but cut-offs ranging from 8 to 21 have been suggested [140, 142] with great variation between populations.

MADRS (Papers II and III)

The Montgomery-Åsberg Depression Rating Scale was specifically developed in 1979 to monitor changes in depressive symptoms in clinical trials [143]. It is a semi-structured interview, which should be conducted by a trained professional. It consists of 10 items regarding the mental aspects of depression. Each can be graded from 0 to 6, 0 being no depressive symptoms and 6 severe symptoms (see appendix 5). The scale is widely used both in trials and as a screening tool in different patient groups, and has been validated in both [144-149]. Originally a cut-off of ≥ 7 was suggested to screen for depression [51], but a score of ≥ 12 is commonly used in psychiatric populations [145]. Furthermore, it varies even more in somatic populations with suggested cut-offs ranging from ≥ 9 to ≥17 [146, 147].

Structured Clinical Interview for DSM-IV Axis I Disorders-Clinician Version (SCID-CV) (Papers II and III)

The Structured Clinical Interview for DSM-IV Axis I Disorders-Clinician Version (SCID-CV) is considered gold standard for diagnosing a major depressive episode according to the DSM-IV criteria [150]. It is a manual for a structured interview which has to be carried out by a trained professional, and in our trial it was performed by either a trained psychology student or psychologist. It does not have a specific time-frame, and the participants can be diagnosed as either having an ongoing episode, being in partial remission from an episode or having had a major depressive episode of mild, moderate or severe degree. Furthermore, it is specified if it is a single MDE or MDE with recurrent episodes.

Seasonal Pattern Assessment Questionnaire (SPAQ) (Paper II)

The Seasonal Pattern Assessment Questionnaire (SPAQ) was designed in 1987 to assess for SAD [151]. It is constructed to measure variation in symptoms and mood during the seasons and consists of six items (mood, sleep, appetite, socializing, weight, energy), which the participants have to grade on a scale from 0-4 (0 being no variation, and 4 maximal variation between seasons), and by adding the scores a Global Seasonality Score (GSS) is calculated. Furthermore, the participants are asked to rate to which degree these variations are a problem to them. A GSS > 10 in combination with a rating of the variations being at least a moderate problem are considered diagnostic for SAD [152]. The questionnaire is widely used, but the reports on validity vary, with some reporting high specificity, sensitivity and internal consistency [153-156], whereas others report rather poor quality as a diagnostic tool [152, 155, 157]. It seems to be adequate for measuring seasonality of all kinds, but insufficient for specific depression diagnostics [158].

Diagnosis of headache (Paper IV)

The diagnosis of headache is based on data from the Tromsø 6 questionnaires. The participants were asked if they had suffered from headache the past year (yes/no), and if the answer was "yes", they were asked to specify if it was of migraine or other type. Further, they were asked about intensity, frequency, duration, additional symptoms, and if there was worsening with physical activity. The questionnaire did not include information about photophobia and number of attacks and therefore did not include all the criteria for diagnosing migraine as defined in the ICHD [84]. In

order to have the most accurate diagnosis of migraine, the participants were only included in our defined migraine group if they had answered "yes" to the migraine question and in addition fulfilled the migraine criteria that we did have information about (duration 4-72 hours and at least two of the following; moderate to severe intensity, pulsating character, unilateral distribution, and worsening with physical activity). Participants, who claimed that they suffered from migraine but did not fill the criteria as stated above, were included in the “non-migraine headache” group and not in the migraine group.

Statistics

In all the papers statistical tests were performed two-sided and a P-value < 0.05 was considered significant. Normal distribution was evaluated visually by inspection of histograms and by determining kurtosis and skewness. In paper I, the main outcome, SCL-10 score, was found not to be normally distributed and was not possible to log transform either. Thus, the SCL-10 score was transformed into a binary outcome, and multiple logistic regression was used in our analyses. Multiple logistic regression was also used in paper IV due to the nature of the primary outcome (headache/migraine yes/no). Independent variables were continuous or categorical as appropriate to their nature. Interaction between relevant variables was tested by inserting an interaction term into the regression analyses and assessing the P-value of the interaction term. In Papers I and IV the interaction term smoking*serum 25(OH)D was not found to be significant, but due to the systematical error of serum 25(OH)D described above, the analyses were performed stratified according to smoking status. No interaction was found between gender and serum 25(OH)D either, but we choose also to present the data stratified according to gender in Paper I, since the women had considerately higher SCL-10 score than men. Adjustment for season was done by dividing serum 25(OH)D into quartiles for each month separately, and then merging the quartiles from each month. Serum 25(OH)D quartiles were used as a categorical variable to calculate OR. Linear trend across the serum 25(OH)D quartiles was tested by first evaluating linearity using orthogonal contrasts, and if a first degree function was found to be most fitting, serum 25(OH)D quartiles were used as a continuous variable in the multiple logistic regression to test for significance.

In Paper II none of the depression scores (BDI-II, HADS or MADRS) were normally distributed and non-parametric statistics were used in the analyses. For comparison of differences between the groups the Mann-Whitney U test was used, and for change within the groups the Wilcoxon signed rank test was used. For comparison of change between the groups, a delta value

was calculated by subtracting the baseline value from the 6 months value, and these delta values were found to normally distributed; thus independent t-test could be used for comparison.

In Paper III internal consistency for each of the depression scales (BDI-II, HADS with subscales and MADRS) were evaluated using Chronbach's α. Internal consistency expressed as Cronbach's α is a measure of how well each factor (i.e. answers to questions) of the questionnaire or interview is correlated with the other factors in the questionnaire [159]. Thus, evaluating how well each of the questions addresses the same condition, i.e. depression. A Cronbach's α above 0.80 is considered a high internal consistency with all the factors (questions) measuring the same condition [159]. Specificity, sensitivity, and Cohen's Kappa at different cut-offs were obtained from cross-tabulation tables. Optimal cut-off values were found by visually inspecting the receiver operating characteristics (ROC) curves where MDE diagnosed by SCID-CV were the gold standard. ROC curves are a visual presentation of sensitivity and specificity, and the area under the curve (AUC) is a measure of how well the test performs better than chance alone (accuracy) [160], where a value of 0.5 is a useless test and 1.0 a perfect test.

In all papers normally distributed data were presented as means and standard deviation, and non-normally distributed data as medians and range (minimum-maximum). In some instances, the latter was also presented as means, because the median was found to be zero and loss of information would occur if only medians were presented.

In all papers proportions were compared with chi-squared test, and means of normally distributed data were compared using paired samples t-test for paired samples and independent t-test for unpaired samples.

All statistical analyses were performed with PASW (SPSS) version 18.0 software for Windows (SPSS Inc., Chicago, Illinois).

Ethics

The participants in the D/Dep study had low serum 25(OH)D levels and were not informed about this until the trial was finished. It could thus be debated, whether it was unethical to treat them with placebo instead of a low dose vitamin D. However, only 11 participants had serum 25(OH)D < 25 nmol/l and only one of these had < 20 nmol/l. Although it cannot be ruled out that they could have benefitted from vitamin D earlier, we do not believe that postponing vitamin D supplement for six months had any serious consequences for the participants' health.

All participants gave written consent before inclusion in the studies. Both the Tromsø Study and D/Dep study were approved by the Regional Committee for Medical and Health Research Ethics. The D/Dep study was in addition approved by the Norwegian Medicines Agency and registered at ClinicalTrials.gov (NTC00960232).

Summary of results

Paper I

In Paper I our aim was to examine the association between serum 25(OH)D level and depressive symptoms in the 12 984 participants in Tromsø 6. After exclusion of participants with missing values in any of the variables used, a total of 10 086 were included in our analyses. Depressive symptoms were assessed using SCL-10 scale based on answers from questionnaires. To differentiate between non-depressed and depressed a SCL-10 score cut-off value of ≥ 1.85 was used. Due to the methodological difficulties with the immunoassay used to measure serum 25(OH)D in smokers, data from smokers and non-smokers were analyzed separately. In crude analyses we found a significant inverse association between serum 25(OH)D level and depressive symptoms ($P < 0.005$) both in smokers and non-smokers. This association remained significant ($P < 0.01$) in both groups after adjusting for known confounders such as age, BMI, season, alcohol consumption, physical activity, glomerular filtration rate (GFR), chronic disease, educational level and marital status. Odds ratio (OR) for the highest serum 25(OH)D quartile compared with the lowest quartile was 0.59 (0.39-0.89) in smokers and 0.74 (0.58-0.95) in non-smokers. The association seemed stronger in women than in men. In summary a significant inverse association was found between serum 25(OH)D level and depressive symptoms, but no conclusions can be drawn with regard to causality due to the cross-sectional design of the study.

Paper II

In Paper II our aim was to confirm the results from Paper I, that there is an association between serum 25(OH)D and depressive symptoms, and in addition to investigate whether supplementation with vitamin D_3 had any effect on depressive symptoms. A total of 357 persons were included on basis of their serum 25(OH)D levels in Tromsø 6. Of these, 243 had low serum 25(OH)D levels (below 55 nmol/l) and the remainder had high serum 25(OH)D levels (above 75 nmol/l) and were recruited as nested controls. The 243 with low serum 25(OH)D levels took part in an intervention study and were randomized to either placebo or 40 000 IU vitamin D pr. week for six months. Depressive symptoms were measured with BDI-II, HADS, MADRS, SCID-CV and GSS. We found the group with low serum 25(OH)D level to have significantly higher HADS and MADRS scores ($P < 0.05$) (more depressed) compared with the ones with high serum 25(OH)D.

Furthermore, the incidence of recurrent depression seemed to be higher in the ones with low serum 25(OH)D levels, but this finding did not quite reach statistical significance (P = 0.056). After six months intervention we found no effect of vitamin D supplementation compared with placebo on any of the depression scores. *Post hoc* analyses of persons with high depression scores showed a possible positive effect of vitamin D compared with placebo in some of the depressions scores, but since it was *post hoc* analyses it must be interpreted with great care. In summary, persons with low serum 25(OH)D have more depressive symptoms, but no convincing effect of vitamin D supplementation was found compared with placebo.

Paper III

The purpose of Paper III was to evaluate the depression scores used in Paper II with regard to internal consistency and psychometric properties, and to find optimal cut-off scores for screening for depression in a healthy population. The data from the study in Paper II were used to perform the analyses. We found that BDI-II, HADS and MADRS all had high area under the ROC curves (0.83-0.87) and high internal consistency (0.75-0.89). Optimal cut-off for screening for MDE was lower than previously reported and was found to be ≥ 12 for BDI-II, ≥ 9 for MADRS, ≥ 10 for HADS total, ≥ 4 for HADS-D, and ≥ 5 for HADS-A resulting in sensitivities ≥ 80% and specificities ≥ 75%. Poor inter-rater agreement was found for all the depression scales (Cohen's kappa 0.20-0.40) indicating that these scales cannot be used for screening for depression, but not diagnosing depression.

Paper IV

The aim of Paper IV was to examine the association between serum 25(OH)D and headache based on data from the Tromsø 6. A total of 11 614 persons were included in the analyses. The diagnosis of headache was based on answers from the questionnaires and was sub-classified into migraine type of headache or non-migraine type of headache. Due to the analytical difficulties with measuring serum 25(OH)D in smokers, the data was analyzed stratified according to smoking status as in Paper I. Three percent of the participants had suffered from migraine the past year and 33% from non-migraine headache. In the unadjusted analyses headache of both types were found to be significantly associated with female gender, younger age, and less alcohol consumption in both smokers and non-smokers. In addition, non-migraine headache was found to be significantly

associated with number of chronic diseases and low level of physical activity, and migraine was found to be positively associated with high educational level. In the non-smoker group both migraine and non-migraine headache were associated with low serum 25(OH)D levels, but no such association was found in smokers. In analyses where adjustment was done for the possible confounders mentioned above in addition to BMI and season, the inverse association between serum 25(OH)D and non-migraine headache remained significant in the non-smokers with an OR of 1.20 (1.04-1.39) in the lowest serum 25(OH)D quartile. In summary, non-migraine type of headache was found to be significantly associated with low serum 25(OH)D levels, but no association was found between migraine and serum 25(OH)D levels.

Methodological considerations

Study design

This thesis is based on data from two cross-sectional studies, one nested case-control study with following randomized controlled intervention design, and one methodological study. Each study type has its strengths and weaknesses. Cross-sectional studies are often community based studies including a considerable part of the population as is also the case with the Tromsø Study, which Papers I and IV are based on. They are frequently used to find associations between diseases and possible explanatory variables, and because of the often large number of participants, even small associations can be found which otherwise would be difficult to establish. Thus, cross-sectional studies can be used to create and support hypotheses about possible disease mechanisms, but since they lack information about temporal relationship, an obligatory factor to be able to establish causality [161], no conclusions about cause/effect can be drawn from this type of study.

Using large study populations heightens the possibility of finding associations. In Paper I we did find an association between serum 25(OH)D and depression, and in Paper IV between serum 25(OH)D and non-migraine headache, and though highly significant, the ORs were relatively moderate (0.74 and 1.20 in non-smokers). This implies that care must be taken when applying these results in a clinical setting, where the associations we found may be of little clinical relevance compared to other risk factors for the diseases in question. But on the other hand, other possible explanatory factors (i.e. alcohol consumption and physical exercise) were of similar magnitude as serum 25(OH)D (results not published), and thus, we find that further investigation of causality was merited.

RCTs are considered the gold standard for establishing causality. However, they do have limitations, especially with external validity which is discussed in detail below. Furthermore, RCTs are often carried out under highly controlled conditions to establish high compliance and include follow-up sessions to heighten adherence to treatment. These factors may make results from RCTs difficult to reproduce in a clinical setting, where compliance and adherence are lower than in a RCT setting. Analyzing from an intension-to-treat perspective in addition to per-protocol, as we did in Paper II, may reduce the difference in adherence between study population and clinical population but will probably not eliminate it completely.

Methodological studies are needed for validating methods used in clinical studies and clinical practice. They are essential in establishing new areas of use for already validated methods, and for validating newly developed methods. In Paper III our main focus was finding optimal cut-off values for diagnosing depression with the different depression scales, and to obtain precise measurements of sensitivity and specificity an appropriate gold standard for comparison was needed. We used the SCID-CV to diagnose depression according to the DSM-IV criteria, the acclaimed gold standard for diagnosing depression [150]. Though often used in diagnosing severe depression, it also contains a graduation of severity as a MDE can be diagnosed as mild, moderate or severe. Thus SCID-CV is also appropriate as a gold standard in our study where the majority of the participants had mild to moderate depressive symptoms.

Intern validity

A study with high intern validity has results that are representative or true for the population that has been studied. Three types of bias can threaten the internal validity: selection bias, information bias, and confounding [162].

Selection bias

Selection bias occur if individuals with different exposure (serum 25(OH)D level) or outcome (depression, headache) have different probability of being included in the study [163]. Papers I and IV are based on data from Tromsø 6 in which selection bias could be caused by non-attendance. The attendance rate was relatively high (67%), but considerate selection bias may have occurred, since persons who participate in population surveys have been reported to have lower mortality rates [122], better general health, and smoke less [164] than those who do not. Furthermore, persons who chose not to participate in Tromsø 6 were more often single [122]. Studies have shown that persons with low levels of serum 25(OH)D have higher mortality rates [165], indicating that the ones not participating might have had lower serum 25(OH)D levels and probably suffering from more chronic diseases as well. Whether this influenced the results from our studies remains speculative, but since non-attendees seem to be in poor health, it is reasonable to assume that they also suffer more from both depression and headache [85, 166]. Thus, if any such bias has occurred it is likely to have attenuated our results, rather than induced non-valid results. A similar problem is related to the higher number of people living alone being non-attendees. Singles have lower serum

25(OH)D level and suffer more often from depression [66], but we assume that this is also more likely to have led to an underestimation of the association, rather than introduced a non-existing relationship.

Selection bias must also be considered in the baseline study in D/Dep study (Paper II and III). In case-control studies the selection of the controls are important with regard to bias. Traditionally case-control studies are done by selecting cases with a positive outcome and comparing them, with regard to exposure, to controls with negative outcome. In our study, we chose a case/control group based on the exposure (low/high serum 25(OH)D) and compared the outcome (depression) between the two groups. The controls were selected randomly from the population with high serum 25(OH)H and matched with regard to gender, age and BMI. There were no differences between the two groups in blood pressure, HbA_1c, fasting blood-glucose or plasma lipids (results not published). Even though selection bias in choosing controls cannot be excluded, it would be random, and we do not believe it would have any effect on our results. In the intervention part of the study selection bias is of less concern, since the randomization process will eliminate any introduced bias.

Information bias

Information bias can occur if measurements or information about the study participants are inaccurate or incorrect, leading to misclassification with regard to exposure and/or outcome groups. Misclassification can be differential or non-differential. In differential misclassification the misclassification in exposure group is dependent on the outcome group, or the misclassification in outcome group is dependent on exposure group. Bias caused by differential misclassification can be difficult to predict, it can lead to falsely exaggerated associations or to falsely underestimated associations. Non-differential misclassification happens when the misclassification is randomly happening in both exposure and outcome groups independently of one another. Non-differential misclassification leads to dilution of the true association [163].

In Papers I and IV questionnaires were used as the primary method to collect data about outcome (depression, headache) and confounders (i.e. smoking, physical exercise, alcohol consumption, chronic diseases). Self-reporting may lead to misclassification, especially information about non-healthy lifestyle habits have been shown to be underreported [167, 168]. Since the participants in Tromsø 6 were not aware of the association investigated, any information bias which may have been introduced by this type of underreporting is likely to be random, and not to be

dependent on neither outcome (depression/headache) nor exposure (serum 25(OH)D), and therefore can be classified as non-differential.

Serum 25(OH)D was measured in Tromsø 6 with the ELICA method by Roche described in the methods section. By overestimating the serum 25(OH)D level in smokers, this could have potentially introduced information bias in all the papers this thesis is based on. In Papers I and IV the association between serum 25(OH)D and depression/headache could be falsely underestimated, since both headache and depression are more common in smokers [93, 169], and the smokers falsely appear to have higher serum 25(OH)D levels. Thus, to avoid this potential differential bias, all analyses in these two papers were performed stratified according to smoking status.

In Papers II and III the participants were recruited based on their serum 25(OH)D level measured in Tromsø 6 as described above. The falsely high serum 25(OH)D levels in smokers could therefore lead to smokers being misclassified to the control group (high serum 25(OH)D level) instead of the case group (low serum 25(OH)D level). Since smokers have higher incidence of depression, this could lead to differential misclassification, and a falsely loss of association between serum 25(OH)D and depressive symptoms at the baseline of the study. At inclusion in the D/Dep study, serum 25(OH)D was measured with a more accurate method, and it was thus possible to adjust for this misclassification by performing baseline analyses with only participants who had truly low (< 55 nmol/l) or high (> 70 nmol/l) serum 25(OH)D. We therefore find it unlikely that differential misclassification has influenced the results from this analysis.

In the intervention part of the study, which only included those with low serum 25(OH)D levels, the falsely high serum 25(OH)D levels in smokers could have led to a relatively high proportion of non-smokers being included in the intervention group. This might have affected the results if non-smokers and smokers were to react differently on vitamin D supplements with regard to depressive symptoms. However, this remains highly speculative and we do not believe this had any significant effect on our results. In Paper III serum 25(OH)D and assignment to case/control group is of no consequence to the analyses, since only depressive symptoms were being studied.

Confounding

Confounding is especially of concern in epidemiological studies like the Tromsø Study, which Papers I and IV were based on. A factor is said to be confounding when it influences both the outcome and the exposure/dependent variable, and thereby possibly creating an association that is

not truly there or leading to an underestimation of a true association [162]. Adjustment for confounding can be done by multiple regression or by stratification.

Confounding is in particular important when studying multifactoral diseases like depression and headache in which lifestyle-related variables influence the risk for developing disease, and the same lifestyle factors also influence the 25(OH)D level. In Paper I we adjusted for age, gender, BMI, alcohol consumption, physical activity, chronic disease, educational level, and marital status, which have previously been described to be associated with both serum 25(OH)D level and depressive symptoms [63, 66]. We also included GFR in our regression analyses since it is related to serum 25(OH)D level [170], but as reported in other studies [62, 63, 65] we did not find an association with depressive symptoms. The rationale for including GFR was as a marker for chronic disease, but since we already included this confounder, this was double adjusting, which could weaken the results and in hindsight should have been avoided. Supplementary regression analyses without adjusting for GFR have shown practically the same results as with GFR included.

In Paper IV we used step-wise introduction of possible confounders, since the literature was found to diverge regarding association between the included factors and headache. Age, gender, chronic diseases and BMI have all been shown to be associated with both headache and serum 25(OH)D [85, 171-173], and were thus included in step 1. Physical exercise, alcohol consumption and educational level have in some studies been found to be associated with headache [89, 174, 175], but in other studies no such association has been found [89, 90]. Therefore these factors were not included until step 2.

In the Tromsø Study assessment of symptoms and blood sampling took place throughout the year, and accordingly, adjusting for time of sampling is important since serum 25(OH)D level varies with season. Several methods exist for adjusting for season, with the most frequently described being the use of "dummy variables". However, it has been shown that adjusting with the use of monthly cutpoints is more accurate and reduces the risk of bias toward the null [176]. Thus, in Papers I and IV adjustment for season was performed by assigning the participants to serum 25(OH)D quartiles for each month separately, and then pooling all the strata.

Adjustment for confounding can also be performed by stratification. As described above, we did stratification for smoking status to overcome the analytical problems with regard to smokers, and not for being a confounder. Had this not been necessary, adjusting for smoking status should have been done by including it as a independent variable in the regression analyses, since smoking

is associated with both headache, depression and serum 25(OH)D [177-179]. Furthermore, in Paper I adjustment for gender was done either by including gender in the regression analyses or by stratification. The interaction term gender*serum 25(OH)D was not significant when introduced into the regression analyses, thus indicating that there was no gender difference in the association between serum 25(OH)D and SCL-10 scores, and thereby implying that stratification was not necessary. Regardless, we did chose to present it stratified as well, since women had significantly higher depression scores and interesting associations might be missed if gender data was pooled. The possible stronger association in women found in our analyses supports this, but caution has to be taken when interpreting the results, since stratification leads to multiple testing with resulting risk for statistical type 1 error.

Extern validity

Selection of the study population is important, both with regard to generalisability of the results and with regard to internal validity. In papers I and IV we used data from Tromsø 6. As described in the methods section, participants were invited to Tromsø 6 based on either their participation in Tromsø 4, or as a random sample from a specific age group in the population. As the Tromsø Study's main aim is to investigate lifestyle related diseases, the majority of the invited persons have been from the higher age groups of the population. For instance in Tromsø 6, only a 10% sample of the population < 40 years were invited (1085 persons), and of these only 47% (509) attended. In the age group 60-79 years everybody was invited and the attendance rate was higher (74%). Whereas it was only 40% (531 persons) in the age group > 80 years where everybody also was invited [122]. This leads to a rather large overrepresentation of the age group 40-79 years compared with the general population, and thus one must be careful if trying to generalize these results also to apply to persons outside this age group.

Since the study, which Papers II and III are based on, was an intervention study, the inclusion criteria were extensive as described in the methods section. This kind of exclusion is common in RCTs, especially if the number of participants is of small to moderate size. There are different reasons for these exclusions; one being to eliminate possible confounders which may make the results difficult to interpret. In this instance persons with recent serious diseases such as coronary heart disease, stroke and cancer were excluded, since these are likely to have low serum 25(OH)D level [2] and are also likely to be depressed [166]. In such participants, an effect of vitamin D on depression might be undetectable since the main reason for depression is the somatic disease and

not low serum 25(OH). Randomization helps to minimize this type of confounding, but in studies with relatively few participants, even a small number of participants as described above could lead to an incorrect negative result. Persons who were using anti-depressants were also excluded. They were likely to have low serum 25(OH)D, but also to have relatively few depressive symptoms due to the treatment. Therefore, they would also have a less pronounced response to vitamin D treatment. Thirdly, persons who were planning a vacation in a sunny place during the study period or were taking vitamin D supplements were excluded to avoid possible vitamin D effect in the placebo group. Lastly, some groups to which high dose vitamin D potentially could be harmful were excluded (pregnant and lactating women, kidney stones, diseases in the parathyroid). Excluding such a large group of persons from the study means that the results can only be generalized to a similar narrowly chosen healthy population. However, the rationale for this study design was first of all to establish if there was any effect of vitamin D on depressive symptoms at all. When having a limited number of participants, this is best done in a very controlled study design. If any effect was found, the next step would be to perform a larger study with a more heterogenic study population to examine if the results would also apply in this type of population.

In Paper III the purpose of the analyses was to investigate the properties of the different depression measurement tools in a healthy population. The results therefore only apply to this fairly healthy group and cannot be generalized to other populations.

Random error

Due to statistical principles, any association or effect found could happen by chance. It is often referred to as random error, and can be dived into type 1 and type 2 error [180]. When finding a significant association or effect, which is not real, it is called a type 1 error. By definition, using a P-value < 0.05 in a statistical analysis means that there is a less than 5% probability that the results were found by chance. Reciprocally, with a P = 0.05 1 in 20 results could be found by chance, and this illustrates that the risk for type 1 error increases with each test done. In Paper II we did *post hoc* analyses on sub-groups stratified according to depression scores, and found that persons who had higher depression scores (HADS and MARDS) had a significant better effect of vitamin D on HADS-score compared with placebo. But since we did not adjust for multiple testing, these results could represent a type 1 error and have to be interpreted with caution.

A type 2 error denominates not finding an association which is really there. Type 2 error can be the result of inadequate number of participants, too short time-frame to see an effect of a given treatment, sub-optimal treatment regime, or otherwise faulty study design. In Papers I and IV a total of more than 10 000 participants were included, probably eliminating any chance of type 2 error. If any association were to be overlooked in such a large population, it would most likely be of little clinical interest. Intervention studies are prone to type 2 error due to small sample sizes, and power calculations are needed to find an appropriate number of participants. In Paper II, change in blood pressure was also a primary outcome and power calculations were originally based on this parameter. However, additional power calculation for MADRS score showed that our study was adequately powered to detect a 20% difference between the groups. A 20% percent difference is considered a clinical relevant change [139]. Power calculation is not possible for the type of statistics performed in Paper III and no consensus about minimal acceptable number of participants has been published. In the original design and validation of the depression scales 100-150 persons have been included [50, 142, 143], which is considerately less than the 357 participants in our study. Thus, we believe our study was adequately powered to produce meaningful results.

General discussion

Association between serum 25(OH)D and depressive symptoms

In Paper I we found serum 25(OH)D to be inversely associated with depressive symptoms in both smokers and non-smokers. This is consistent with results from earlier studies [62-64, 68-72], and though the magnitude of the association is difficult to compare due to methodological differences, the ORs reported seem to be in the same range (OR 0.46-0.92) [70, 71] as our results. However, some other studies did not find an association [66, 67, 73], and though it is possible that this represents a true difference between populations studied, it is plausible that methodological differences may be the cause of this divergence. Especially the size of the study population is of importance; the three studies which did not find an association were relatively small ranging from 118 to 3916 participants [66, 73] as compared to studies which did find an association with study populations ranging from 954 to 12 594 [65, 71]. In addition, the three negative studies did all report a trend towards higher level of depressive symptoms with low serum 25(OH)D levels, but they did not reach significance, which further supports the assumption that adequate study size is needed to verify the association.

An additional cause for the diverging results could be the different methods used for measuring depression and number and type of confounders adjusted for as previously described in the introduction. The majority of studies adjust for age, BMI, gender, smoking status, marital status, educational level/income, season, physical activity, and chronic diseases as we did in Paper I. Further adjustments seem to be of minor importance, since studies which adjust for a wide variety of confounders such as altitude [68], urbanization [64], MMSE score [65], and adverse life events [63] all report a significant association. Furthermore, whether serum 25(OH)D was used as a continuous variable [71], divided into quartiles [62, 68], tertiles [64] or dichotomized [65] did not seem to influence results either.

Different methods for assessing depression might affect the results as well. None of the studies mentioned above used clinical interviews for diagnosing depression, and a variety of different questionnaires have been used. The most frequently used being the CES-D, which was used in both studies which found an association [62, 65, 71, 72], and in one that did not find an association [67]. Thus, the diverging results do not appear to be dependent on questionnaires used,

although most of the questionnaires have only been used in single studies and trends might be difficult to notice.

Lastly, there could be different associations between depression and vitamin D in different populations. However, negative results as well as positive were reported from both the USA and China [66, 67, 70, 71]. Thus, no geographical influence seems to be evident. Similarly, reported associations from a wide array of age-groups [62, 64, 69, 71] indicate that the association seems to be present in all stages of life.

In summary, the evidence for an association between serum 25(OH)D and depressive symptoms is convincing and our results support these findings. However, the risk of confounding is apparent and causality cannot be established from cross-sectional studies. Furthermore, the moderate ORs reported, and the need for large study samples to obtain significant results, indicate that the impact of vitamin D in relation to depressive symptoms might be of limited clinical importance at a population level. However, the prevalence of depression in the investigated populations was low, and the association may be stronger in selected populations with higher prevalence of depression.

Effect of vitamin D on depressive symptoms

In Paper II, our results from the case-control part of the study support the findings reported in Paper I, with significantly higher depression scores (HADS and MADRS) in the cases with low serum 25(OH)D compared with the controls with high serum 25(OH)D. However, we did not find any effect of vitamin D supplements on depressive symptoms compared with placebo.

As mentioned earlier, only a few studies have investigated the effect of vitamin D on depressive symptoms, and they all have methodological problems and the results vary. Three studies used additional supplements such as calcium [77, 80] and vitamin A [76] together with vitamin D making it impossible to establish which of the supplements caused the positive effect reported in two of the studies [76, 77]. Some did in addition use small doses of vitamin D (200 IU/day) for a short duration of time (5-9 days) [76, 77]. Since it has been shown that 400 IU vitamin D/day for eight weeks only raises serum 25(OH)D 11 nmol/l [181], it is highly unlikely that such small doses for a short duration of time would have any effect. Another difficulty is illustrated in the study by *Vieth et al.* [74] where a positive effect of 4000 IU vitamin D daily compared to 600 IU daily was found in the first part of the study, but the results could not be reproduced when the

study was performed again. Supplying the participants with a relatively low dose of vitamin D instead of placebo due to ethical considerations as Vieth did, makes the results very hard to interpret. This is especially important in negative studies like the Women's Health Initiative [80] where the participants were allowed to continue with supplements up to 600 IU/day, and in the study on 2258 women reported by *Sanders et al.* [75] where an additional daily dose up to 400 IU was accepted. A dose of 600 IU/day is above the recommended dose in Scandinavia [9] and in line with the US recommendations [29], thus one would expect the effect of additional vitamin D supplementation to be rather limited.

Only three well-designed RCTs have been reported so far, of which two were in non-clinical populations comparable to our study population. *Dean et al.* [79] did not find any effect on BDI score of 5000 IU vitamin D/day for six weeks compared to placebo in a RCT on 128 healthy volunteers. Though the time frame of six weeks is considered short in a depression perspective (MDE median duration three months [182]) it should be adequate to detect alleviation of symptoms if the treatment was effective. However, although the authors state sufficient power regarding the primary outcome, BDI score was a secondary outcome and the study seemed under-powered in relation to BDI scores. This suggests a type 2 statistical error as an explanation for the results rather than a true negative finding. On the other hand, *Jorde et al.* [78] did find an effect of vitamin D 20 000-40 000 IU/week (two treatments groups pooled) compared with placebo on the cognitive-affective subscale of BDI in 334 obese persons when treated for one year. There was however no effect on total BDI score, and the effect was modest, implying that that the results might be of relatively small clinical importance. Contrary to this, *Khoraminya et al.* [81] reported a stunning effect of vitamin D 1500 IU/day in addition to Fluxetine 20 mg (a selective serotonin reuptake inhibitor) compared with Fluxetine 20 mg alone in clinically depressed patients. The study was small with only 20 patients in each treatment arm and they had relatively high serum 25(OH)D levels ≈ 23 ng/ml (approximately 57 nmol/l) at inclusion. In accordance with the population studies mentioned above, persons with such high serum 25(OH)D would be in the "low risk/highest serum 25(OH)D quartile" category with regard to depression, and the strong response reported here seems counterintuitive. In addition, with an effect this pronounced one would expect differences in effect of anti-depressants according to season, but no such reports have been found. Accordingly, further research are needed to confirm these results.

Our study in Paper II did not have any of the methodological difficulties discussed above, but we could not confirm the results from *Jorde et al.* [78], and other aspects causing a negative result

must be considered. Due to ethical considerations, persons with high depression scores were excluded from the intervention part of the study, thus only persons with none to moderate depressive symptoms were included. Though some authors state that sub-clinical depression cannot be used for understanding of major depressive disorder [183], there is growing consensus that depression is to be considered a continuum of symptoms [184]. This implies that a mild to moderately depressed population is adequate for studying treatment effects in major depressive disorder. However, the placebo effect in mildly depressed persons have been shown to be of similar magnitude as anti-depressants [185], which may explain why we did not find an effect of vitamin D. On the other hand, since the association found in the population studies were moderate, it is likely to assume that an effect of vitamin D on depressive symptoms would be small and indistinctive compared with cognitive therapy and anti-depressants, which would be obligatory in a trial with severely depressed patients. Therefore trials on mild to moderately depressed persons are important.

An additional consideration is that the participants in the intervention study were recruited based on assumed low serum 25(OH)D. However, the mean serum 25(OH)D was 47.3 nmol/l, which though lower than the population average, is close to recommended values [23]. Even though the participants with low serum 25(OH)D did have higher depression scores, persons with near normal serum 25(OH)D might be less prone to respond to vitamin D treatment, thus attenuating the effect compared with placebo.

SAD is of particular interest in Tromsø due to the location far north, and in Paper II we included the GSS calculated from the SPAQ to assess for SAD. We did not find any association neither at baseline nor after intervention, but the results must be interpreted with caution. In hindsight, neither the diagnosis of SAD nor the SPAQ are well validated as summarized by *Hansen et al.* [38]. In addition the SPAQ is retrospective which makes it unsuitable for intervention studies. Furthermore, the study only lasted six months, a time-frame too short to notice any change in seasonality. Thus, no conclusions can be drawn with regard to depression with a seasonal pattern and vitamin D.

In summary, in Paper II we did not find any effect of vitamin D supplementation on depressive symptoms compared with placebo in a study population with relatively low serum 25(OH)D levels. Our results does not confirm the findings of previous studies [78, 81], even though our study was adequately powered and included persons with relatively low serum 25(OH)D to optimize possible treatment effect. The negative result could represent true lack of effect, but our

post hoc analyses suggest a positive effect in persons with high depression scores, thus supporting the results found by *Khoraminya et al.* [81]. Nonetheless, these analyses must be interpreted carefully and further studies in preferably clinically depressed persons with low serum 25(OH)D are needed to investigate this effect.

Validation of depression instruments

In Paper III we evaluated the BDI-II, MADRS and HADS with regard to internal consistency, correlation between scales, specificity, sensitivity, inter-rater agreement and suggested cut-off points for our study population based on a DSM-IV diagnosis of depression.

The internal validity of HADS is well validated in a variety of somatic populations, primary care [140, 141], and general populations [186, 187]. Though, only one study has used a clinical interview as gold standard for investigating HADS's ability as screening instrument for depression in a general population [188]. These authors reported a sensitivity of 90% and specificity 91% for screening for depression when using a cut-off of > 8 on HADS-D, but did not report any other parameters. Our results with a suggested cut-off of HADS-D >3 (sensitivity 85%, specificity 79%) and HADS-total > 9 (sensitivity 80%, specificity 84%) does not support these findings. On the other hand, one study on a primary care population found results almost identical to ours [189], whereas two others found higher cut-off values with suggested HADS-D > 6 [190] and > 8 [191]. One other study from primary care reported a cut-off for HAD of > 8 [142], but it was not specified if it was HADS-total or HADS-D. Even though *Bjelland et al* [140] reported it to be HADS-total in their review, caution is needed due to lack of detail.

Similar to HADS, the BDII-II has been validated with regard to internal consistency in several populations, such as college students [192], psychiatric out-patients [133], primary care [134], and somatic patients [193], but no studies have so far examined the properties of BDI-II in a general population. Two studies from primary care using diagnostic interviews as gold standard for depression found diverging results; *Arnau et al.* [194] suggested a cut-off > 18 for depression with sensitivity of 94% and specificity of 92%, whereas *Dutton et al.* [195] found a cut-off of > 14 to yield maximal sensitivity and specificity. We found optimal screening potential with a BDI-II > 13 with sensitivity and specificity in the mid-eighties, thus lower than reported in primary care, but not as diverging as seen with HADS.

MADRS is also widely used and validated in different populations like somatic [148, 196], the elderly [146, 147], and psychiatric patients [197]. On the other hand, no studies on MADRS's properties in a general population have been published. In a review *Zimmermann et al.* [144] estimated a cut-off for complete lack of clinical significant depressive symptoms to be > 5, and if using a broader approach to being "depression free" they found a cut-off of > 10. These findings were, however, based on standard deviations from a population assumed to be without depression, and no diagnostic interviews were performed. Our results suggest a MADRS cut-off > 10 (sensitivity 80%, specificity 86%), which is consistent with Zimmermann's findings but are somewhat lower than the cut-off of > 13 that originally was suggested by *Snaith et al.* [51]. Furthermore, optimal cut-offs ranging from > 8 to > 18 have been suggested based on other study populations [146, 149].

Overall, our results are comparable to previous studies with regard to internal consistency, correlation between the different scores [140, 198], sensitivity, and specificity. However, the cut-offs we found were lower than reported in other study populations, suggesting that persons from a healthy study population like ours can be clinically depressed even though they have low depression scores. One possible explanation for this might be that the participants were very healthy compared with the general population or a primary care population. Persons participating in studies have been shown to be more healthy than those who do not [164], and since our study participants had completed at least one of the Tromsø Study surveys and in addition had not met any of our exclusion criteria for the D/Dep study, they were doubtlessly more healthy than the population in general. Healthy persons have been shown to cope better with stressful situations and report fewer depressive symptoms [199]. Their ability to cope better might be reflected in their more modest report of depressive symptoms as compared with persons suffering from the same level of depression but with poorer coping skills (i.e. a general population or primary care population). This might explain the relatively low cut-offs we found.

Another possible explanation for the low cut-offs for diagnosing depression, could be presence of atypical symptoms (e.g. sensitivity to rejection, fear of rejection, heavy/leaden feeling in the extremities) which are poorly measured with the depressions scales, thus resulting in misleadingly low scores. However, no reports of these symptoms being more prevalent in presumed healthy populations were found, but since our number of persons with MDE was small, these symptoms could be overrepresented by pure chance.

In addition, only persons with either high or low serum 25(OH)D were included, and it could be speculated if the missing "middle group" might affect the results, since vitamin D might be associated with depression. However, the differences between the groups were small, even overlapping, and it is unlikely that persons with different serum 25(OH)D levels would have responded differently to depression questionnaires.

In summary, we found the BDI-II, HADS and MADRS to be instruments with high internal consistency and concurrent validity. Cut-off scores for diagnosing a MDE were lower than previously reported in other population types. However, similar sensitivity and specificity were found, indicating that all the instruments investigated were adequate for screening purposes given that appropriate cut-offs are used. The inter-rater agreement was poor for all instruments, confirming reports from previous studies about poor diagnostic qualities.

Association between serum 25(OH)D and headache

In Paper IV we did find an inverse association between serum 25(OH)D and non-migraine headache, but no association was found between serum 25(OH)D and migraine. These findings are supportive of the results reported by *Knutsen et al.* [5], who found higher incidence of vitamin D deficiency in patients with headache compared with patients with other types of pain, although a considerable level of confounding probably influenced the results in that study. Our findings are also supportive of the theory by *Prakash et al.* [6] of headache being associated with latitude. Their study was, however, completely unadjusted and no measurements of vitamin D were included, thus it might as well indicate an association between westernized lifestyle and headache or coincidence as noted by *Yang et al.* [112].

The non-migraine headache in our study is assumed to be TTH, since the prevalence of other types such as cluster headache has been reported to be low [200]. TTH is thought to be multifactoral with several possible disease mechanisms and predisposing factors. In our study we did adjust for as many of the known factors as possible which were also believed to be confounders in relation to vitamin D. However, some possible confounders such as weather, personality traits, stress level, and sleep disturbances [89, 90, 175] which have been described to be associated with headache are most likely also influencing vitamin D levels, but they were not included in our analyses. Thus, even though extensive adjustments were performed, the association between serum 25(OH)D might be explained by confounding and no conclusions about causality can be drawn.

Only one intervention study about vitamin D and TTH has been published [115]. Eight patients with osteomalacia and chronic TTH were treated with vitamin D 1000-1500 IU/day and 1-1.5g calcium/day for varying amounts of time, and they all had positive effect on headache symptoms. However, the study was not placebo controlled, and the participants received additional calcium which could possibly also have an effect on headache. In addition, the alleviation of headache symptoms might be related to treatment of the condition causing the headache (osteomalacia). Thus, well designed RCTs are needed to investigate the association further.

We did not find an association between low serum 25(OH)D and migraine, which is in accordance with reported higher incidence of migraine attacks during spring and summer-time [109, 201, 202]. The results are not supportive of an effect of vitamin D in patients suffering from migraine as reported by *Thys-Jacobs* [116, 117], but these were uncontrolled case-studies and cannot be given much consideration. One limitation of our study was the strict criteria for defining migraine, thus the number of migraineurs were relatively small with only 322 persons assigned to the migraine category. However, accurate classification was important since misclassification of persons with TTH to the migraine group would lead to a false association. Moreover, no convincing signs of a trend across the serum 25(OH)D quartiles could be seen, and we believe that our findings are not a result of an under-powered study but a truly lacking association.

In summary we did find an association between TTH and serum 25(OH)D, but even though adjustments were done for confounders, this association might represent a general unhealthy lifestyle leading to low serum 25(OH)D in headache sufferers instead of a causal relationship. No association was found between migraine and serum 25(OH)D.

Conclusions and implications

- In accordance with previous studies, we found a significant inverse association between serum 25(OH)D and depressive symptoms measured with SCL-10 in the sixth survey of the Tromsø Study. Causality cannot be established from this study type and intervention studies are needed to obtain further information about the relationship between vitamin D and depression. Consistent findings of serum 25(OH)D below recommended levels in depressed persons imply that routine serum 25(OH)D screening or treatment with vitamin D should be considered in persons suffering from depression to alleviate other potential harmful effects of vitamin D insufficiency.

- Our results do not support a beneficial effect of vitamin D supplements on depressive symptoms in a population with low serum 25(OH)D. The lack of effect might be explained by the placebo-effect overshadowing a true effect of vitamin D in mildly depressed participants. *Post hoc* analyses from our study indicate that persons with more pronounced depressive symptoms might have beneficial effect of vitamin D. This is consistent with results in another RCT, and thus, further studies in clinically depressed persons are needed to confirm these findings.

- We found BDI-II, HADS and MADRS to have high internal consistency, moderate to high concurrent validity and poor inter-rater agreement in our healthy study population. The results are similar to what have been reported in several other populations, and confirm that the instruments are of solid construct but have poor quality as diagnostic tools for MDE.

- Optimal cut-off values for diagnosing depression in our healthy study population were found to be > 9 for HADS, > 10 for MADRS and > 13 for BDI-II, all resulting in sensitivity and specificity above 80%. These cut-offs are lower than what have been reported in other population, and might reflect that healthy persons have better coping abilities and report lower symptom level. The results imply that care must be taken when using these instruments to screen for depression in a healthy population, due to risk of low sensitivity if traditional cut-offs are used. In addition, further studies in similar populations are needed to confirm these results.

- We found a significant inverse association between serum 25(OH)D and TTH in the sixth survey of the Tromsø Study. Though adjustments were performed for numerous confounders, several potential confounders were not included, and the results might reflect lifestyle in headache patients rather than a true association. Thus, further studies, both population studies and intervention studies, are needed. Our results do not support any association between migraine and serum 25(OH)D.

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Paper 1

Paper 2

Paper 3

Paper 4

Errata

Paper II.

In the second column of Table 1, the female/male ration should read 133/110, not 123/110. In the same column the range for BDI-total should read 0-52, not 0-49.

In Table 2, second column the BDI-total range should read 0-52, not 0-49.

Paper IV.

Page 1504, first column, second paragraph, citation with number 15 should read 25.

Appendix

The Tromsø Study

The form will be read electronically. Please use a blue or black pen
You can not use comas, use upper-case letters.

2007 – 2008 Confidential

HEALTH AND DISEASES

1 **How do you in general consider your own health to be?**

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

2 **How is your health compared to others in your age?**

- ☐ Much better
- ☐ A little better
- ☐ About the same
- ☐ A little worse
- ☐ Much worse

3 **Do you have, or have you had?**

	Yes	No	Age first time
A heart attack	☐	☐	
Angina pectoris *(heart cramp)*	☐	☐	
Cerebral stroke/brain hemorrhage	☐	☐	
Atrial fibrillation	☐	☐	
High blood pressure	☐	☐	
Osteoporosis	☐	☐	
Asthma	☐	☐	
Chronic bronchitis/Emphysema/COPD	☐	☐	
Diabetes	☐	☐	
Psychological problems *(for which you have sought help)*	☐	☐	
Hypothyroidism	☐	☐	
Kidney disease, *not including urinary tract infection (UTI)*	☐	☐	
Migraine	☐	☐	

4 **Do you have persistent or constantly recurring pain that has lasted for 3 months or more?**

☐ Yes ☐ No

5 **How often have you suffered from sleeplessness during the last 12 months?**

- ☐ Never, or just a few times
- ☐ 1-3 times a month
- ☐ Approximately once a week
- ☐ More that once a week

6 **Below you find a list of various problems. Have you experienced any of this during the last week (including today)?** (Tick once for each complaint)

	No complaint	Little complaint	Pretty much	Very much
Sudden fear without reason	☐	☐	☐	☐
Felt afraid or anxious	☐	☐	☐	☐
Faintness or dizziness	☐	☐	☐	☐
Felt tense or upset	☐	☐	☐	☐
Tend to blame yourself	☐	☐	☐	☐
Sleeping problems	☐	☐	☐	☐
Depressed, sad	☐	☐	☐	☐
Feeling of being useless, worthless	☐	☐	☐	☐
Feeling that everything is a struggle	☐	☐	☐	☐
Feeling of hopelessness with regard to the future	☐	☐	☐	☐

USE OF HEALTH SERVICES

7 **Have you during the last 12 months visited:**
If YES; how many times?

	Yes	No	No. of times
General practitioner (GP)	☐	☐	
Psychiatrist/psychologist	☐	☐	
Medical specialist outside hospital *(other than general practitioner/psychiatrist)*	☐	☐	
Physiotherapist	☐	☐	
Chiropractor	☐	☐	
Alternative practitioner *(homeopath, acupuncturist, foot zone therapist, herbal medicine practitioner, laying on hands practitioner, healer, clairvoyant, etc.)*	☐	☐	
Dentist/dental service	☐	☐	

8 **Have you during the last 12 months been to a hospital?**

	Yes	No	No. of times
Admitted to a hospital	☐	☐	
Had consultation in a hospital without admission;			
At psychiatric out-patient clinic	☐	☐	
At another out-patient clinic	☐	☐	

9 **Have you undergone any surgery during the last 3 years?**

☐ Yes ☐ No

USE OF MEDICINES

10 **Do you currently use, or have you used some of the following medicines?** (Tick once for each line)

	Never used	Now	Earlier	Age first time
Blood pressure lowering drugs	☐	☐	☐	
Cholesterol lowering drugs	☐	☐	☐	
Drugs for heart disease	☐	☐	☐	
Diuretics	☐	☐	☐	
Drugs for osteoporosis	☐	☐	☐	
Insulin	☐	☐	☐	
Tablets for diabetes	☐	☐	☐	
The drugs for hypothyroidism Thyroxine/levaxin	☐	☐	☐	

11 **How often have you during the last 4 weeks used the following medicines?** (Tick once for each line)

	Not used in the last 4 weeks	Less than every week	Every week, but not daily	Daily
Painkillers on prescription	☐	☐	☐	☐
Painkillers non-prescription	☐	☐	☐	☐
Sleeping pills	☐	☐	☐	☐
Tranquillizers	☐	☐	☐	☐
Antidepressants	☐	☐	☐	☐

12 **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 vitamins, minerals, herbs, natural remedies, other nutritional supplements, etc.

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

When attending you will be asked whether you have used antibiotics or painkillers the last 24 hours. If you have, you will be asked to provide the name of the drug, strength, dose and time of use.

FAMILY AND FRIENDS

13 **Who do you live with?** (Tick for each question and give the number)

	Yes	No	Number
Spouse/partner	☐	☐	
Other people older than 18 years	☐	☐	
People younger than 18 years	☐	☐	

14 **Tick for the relatives who have or have had**

	Parents	Children	Siblings
A heart attack	☐	☐	☐
A heart attack before age of 60	☐	☐	☐
Angina pectoris *(heart cramp)*	☐	☐	☐
Cerebral stroke/brain haemorrhage	☐	☐	☐
Osteoporosis	☐	☐	☐
Gastric/duodenal ulcers	☐	☐	☐
Asthma	☐	☐	☐
Diabetes	☐	☐	☐
Dementia	☐	☐	☐
Psychological problems	☐	☐	☐
Substance abuse	☐	☐	☐

15 **Do you have enough friends who can give you help when you need it?**

☐ Yes ☐ No

16 **Do you have enough friends whom you can talk confidentially with?**

☐ Yes ☐ No

17 **How often do you normally take part in organised gatherings, e.g. sport 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

WORK, SOCIAL SECURITY AND INCOME

18 **What is the highest level of education you have completed?** (Tick once)

☐ Primary/secondary school, modern secondary school
☐ Technical school, vocational school, 1-2 years senior high school
☐ High school diploma
☐ College/university less than 4 years
☐ College/university 4 years or more

19 **What is your main activity?** (Tick once)

☐ Full time work ☐ Housekeeping
☐ Part time work ☐ Retired/benefit recipient
☐ Unemployed ☐ Student/military service

20 **Do you receive any of the following benefits?**

- ☐ Old-age, early retirement or survivor pension
- ☐ Sickness benefit (on sick leave)
- ☐ Rehabilitation benefit
- ☐ Full disability pension
- ☐ Partial disability pension
- ☐ Unemployment benefits
- ☐ Transition benefit for single parents
- ☐ Social welfare benefits

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

- ☐ Less than 125 000 NOK
- ☐ 125 000-200 000 NOK
- ☐ 201 000-300 000 NOK
- ☐ 301 000-400 000 NOK
- ☐ 401 000-550 000 NOK
- ☐ 551 000-700 000 NOK
- ☐ 701 000 -850 000 NOK
- ☐ More than 850 000 NOK

22 **Do you work outdoor at least 25% of the time, or in cold buildings (e.g. storehouse/industry buildings)?**

- ☐ Yes
- ☐ No

PHYSICAL ACTIVITY

23 **If you have paid or unpaid work, which statement describes your work best?**

- ☐ 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. postman, nursing, construction)*
- ☐ Heavy manual labour

24 **Describe your exercise and physical exertion in leisure time. If your activity varies much, e.g. between summer and winter, then give an average. The question refers only to the last year.** (Tick the most appropriate box)

- ☐ Reading, watching TV, or other sedentary activity.
- ☐ Walking, cycling, or other forms of exercise at least 4 hours a week *(include walking or cycling to work, Sunday-walk/stroll, etc.)*
- ☐ Participation in recreational sports, heavy gardening, etc. *(note:duration of activity at least 4 hours a week)*
- ☐ Participation in hard training or sports competitions, regularly several times a week.

25 **How often do you exercise?** (With exercise we mean for example walking, skiing, swimming or training/sports)

- ☐ Never
- ☐ Less than once a week
- ☐ Once a week
- ☐ 2-3 times a week
- ☐ Approximately every day

26 **How hard do you exercise on average?**

- ☐ Easy- do not become short-winded or sweaty
- ☐ You become short-winded and sweaty
- ☐ Hard- you become exhausted

27 **For how long time do you exercise every time on average?**

- ☐ Less than 15 minutes
- ☐ 15-29 minutes
- ☐ 30-60 minutes
- ☐ More than 1 hour

ALCOHOL AND TOBACCO

28 **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

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

- ☐ 1-2
- ☐ 3-4
- ☐ 5-6
- ☐ 7-9
- ☐ 10 or more

30 **How often do you drink 6 units of alcohol or more in one occasion?**

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

31 **Do you smoke sometimes, but not daily?**

- ☐ Yes
- ☐ No

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

- ☐ Yes, now
- ☐ Yes, previously
- ☐ Never

33 **If you previously smoked daily, how long is it since you quit?**

Number of years ☐☐

34 **If you currently smoke, or have smoked previously: How many cigarettes do you or did you usually smoke per day?**

Number of cigarettes ☐☐

35 **How old were you when you began daily smoking?**

Age in years ☐☐

36 **How many years in all have you smoked daily?**

Number of years ☐☐

37 **Do you use or have you used snuff or chewing tobacco?**

- ☐ No, never
- ☐ Yes, previously
- ☐ Yes, sometimes
- ☐ Yes, daily

DIET

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

☐ Yes ☐ No

39 **How many units of fruit or vegetables do you eat on average per day?** (units means for example a fruit, a cup of juice, potatoes, vegetables)

Number of units ☐

40 **How many times a week do you eat warm dinner?**

Number ☐

41 **How often do you usually eat these foods?**
(Tick once for each line)

	0-1 times/ mth	2-3 times/ mth	1-3 times/ week	4-6 times/ week	1-2 times/ day
Potatoes	☐	☐	☐	☐	☐
Pasta/rice	☐	☐	☐	☐	☐
Meat *(not processed)*	☐	☐	☐	☐	☐
Processed meat *(sausages, hamburger, etc.)*	☐	☐	☐	☐	☐
Fruits, vegetables, berries	☐	☐	☐	☐	☐
Lean fish	☐	☐	☐	☐	☐
Fatty fish *(e.g. salmon, trout, mackerel, herring, halibut, redfish)*	☐	☐	☐	☐	☐

42 **How much do you usually drink the following?**
(Tick once for each line)

	Rarely/ never	1-6 glasses /week	1 glass /day	2-3 glasses /day	4 or more glasses /day
Milk, curdled milk, yoghurt	☐	☐	☐	☐	☐
Juice	☐	☐	☐	☐	☐
Soft drinks with sugar	☐	☐	☐	☐	☐

43 **How many cups of coffee and tea do you drink daily?** (Put 0 for the types you do not drink daily)

	Number of cups
Filtered coffee	☐
Boiled coffee (coarsely ground coffee for brewing)	☐
Other types of coffee	☐
Tea	☐

44 **How often do you usually eat cod liver and roe?**
(i.e. "mølje")

☐ Rarely/never ☐ 1-3 times/year ☐ 4-6 times/year
☐ 7-12 times/year ☐ More than 12 times/year

45 **Do you use the following nutritional supplements?**

	Daily	Sometimes	No
Cod liver oil or fish oil capsules	☐	☐	☐
Omega 3 capsules *(fish oil, seal oil)*	☐	☐	☐
Calcium tablets	☐	☐	☐

QUESTIONS FOR WOMEN

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

☐ Yes ☐ No ☐ Uncertain

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

Number ☐

48 **If you have given birth, fill in for each child: birth year, birth weight and months of breastfeeding** (Fill in the best you can)

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

49 **Have you during pregnancy had high blood pressure?**

☐ Yes ☐ No

50 **If yes, during which pregnancy?**

☐ The first ☐ Second or later

51 **Have you during pregnancy had proteinuria?**

☐ Yes ☐ No

52 **If yes, during which pregnancy?**

☐ The first ☐ Second or later

53 **Were any of your children delivered prematurely (a month or more before the due date) because of preeclampsia?**

☐ Yes ☐ No

54 **If yes, which child?**

1st child	2nd child	3rd child	4th child	5th child	6th child
☐	☐	☐	☐	☐	☐

55 **How old were you when you started menstruating?**

Age ☐

56 **Do you currently use any prescribed drug influencing the menstruation?**

Oral contraceptives, hormonal intrautrine or similar ☐ Yes ☐ No

Hormone treatment for menopausal problems ☐ Yes ☐ No

When attending you will get supplementary questions about menstruation and any use of hormones. Write down on a sheet of paper the names of all the hormones you have used and bring it with you. You will also be asked whether your menstruation have ceased and possibly when and why.

1. DESCRIPTION OF YOUR HEALTH STATUS

By placing a tick in one box in each group below, please indicate which statements best describe your own health state today:

1.01 **Mobility**

- ☐ I have no problems in walking about
- ☐ I have some problems in walking about
- ☐ I am confined to bed

1.02 **Self-care**

- ☐ I have no problems with self-care
- ☐ I have some problems washing or dressing myself
- ☐ I am unable to wash or dress myself

1.03 **Usual activities** (e.g. work, study, housework, family or leisure activities)

- ☐ I have no problems with performing my usual activities
- ☐ I have some problems with performing my usual activities
- ☐ I am unable to perform my usual activities

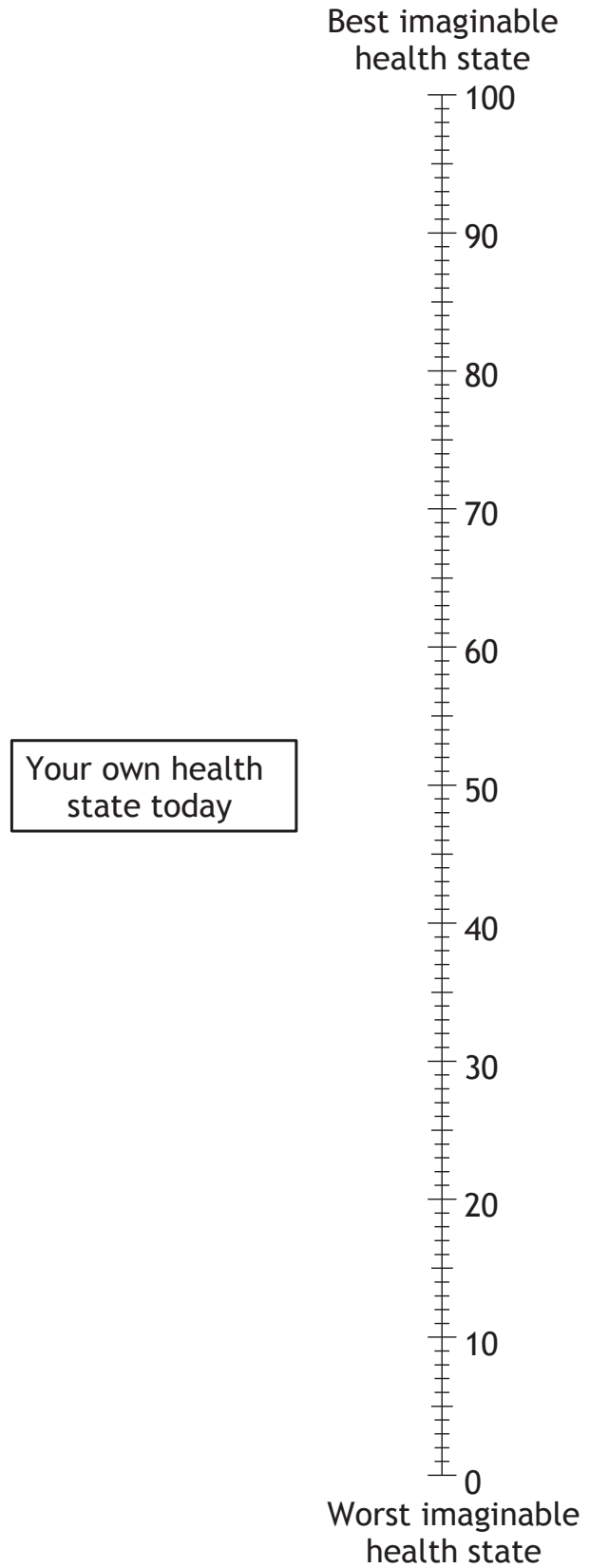
1.04 **Pain and discomfort**

- ☐ I have no pain or discomfort
- ☐ I have moderate pain or discomfort
- ☐ I have extreme pain or discomfort

1.05 **Anxiety and depression**

- ☐ I am not anxious or depressed
- ☐ I am moderately anxious or depressed
- ☐ I am extremely anxious or depressed

1.6 To allow you to show us how good or bad your state of health is we have made a scale (almost like a thermometer) where the best state of health you can imagine is marked 100 and the worst 0. We ask you to show your state of health by drawing a line from the box below to the point on the scale that best fits your state of health.



2. CHILDHOOD/YOUTH AND AFFILIATION

2.01 **Where did you live at the age of 1 year?**

- ☐ In Tromsø *(with present municipal borders)*
- ☐ In Troms, but not Tromsø
- ☐ In Finnmark
- ☐ In Nordland
- ☐ Another place in Norway
- ☐ Abroad

2.02 **How was your family's financial situation during your childhood?**

- ☐ Very good
- ☐ Good
- ☐ Difficult
- ☐ Very difficult

2.03 **What is the importance of religion in your life?**

- ☐ Very important
- ☐ Somewhat important
- ☐ Not important

2.04 **What do you consider yourself as?** (Tick for one or more alternatives)

- ☐ Norwegian
- ☐ Sami
- ☐ Kven/Finnish
- ☐ Another

2.05 **How many siblings and children do you have/have you had?**

Number of siblings ..... ☐☐

Number of children ..... ☐☐

2.06 **Is your mother alive?**

☐ Yes ☐ No

If NO: her age when she died ..... ☐☐

Is your father alive?

☐ Yes ☐ No

If NO: his age when he died ..... ☐☐

2.07 **What was/is the highest completed education for your parents and your spouse/partner?** (Tick once for each column)

	Mother	Father	Spouse/ partner
7-10 years primary/secondary school, modern secondary school	☐	☐	☐
Technical school, vocational school, 1-2 years senior high school	☐	☐	☐
High school diploma	☐	☐	☐
College or university (less than 4 years)	☐	☐	☐
College or university (4 years or more)	☐	☐	☐

3. WELL BEING AND LIVING CONDITIONS

3.01 **Below are three statements about satisfaction with life as a whole. Then there are two statements about views on your own health. Show how you agree or disagree with each of the statements by ticking in the box for the number you think fits best for you.** (tick once for each statement)

	Completely disagree 1	2	3	4	5	6	7 Completely agree
In most ways my life is close to my ideal	☐	☐	☐	☐	☐	☐	☐
My life conditions are excellent	☐	☐	☐	☐	☐	☐	☐
I am satisfied with my life	☐	☐	☐	☐	☐	☐	☐
I have a positive view of my future health	☐	☐	☐	☐	☐	☐	☐
By living healthy, I can prevent serious diseases	☐	☐	☐	☐	☐	☐	☐

3.02 **Below are four statements concerning your current job conditions, or if you are not working now, the last job you had.** (Tick once for each statement)

	Completely disagree 1	2	3	4	5	6	7 Completely agree
My work is tiring, physically or mentally	☐	☐	☐	☐	☐	☐	☐
I have sufficient influence on when and how my work should be done	☐	☐	☐	☐	☐	☐	☐
I am being bullied or harassed at work	☐	☐	☐	☐	☐	☐	☐
I am being treated fairly at work	☐	☐	☐	☐	☐	☐	☐

3.03 **I consider my occupation to have the following social status in the society** (if you are not currently employed, think about your latest occupation)

- ☐ Very high status
- ☐ Fairly high status
- ☐ Medium status
- ☐ Fairly low status
- ☐ Very low status

3.04 **Have you over a long period experienced any of the following?** (Tick one or more for each line)

	No	Yes, as a child	Yes, as adult	Yes, last year
Been tormented, or threatened with violence	☐	☐	☐	☐
Been beaten, kicked at or victim of other types of violence	☐	☐	☐	☐
Someone in your close family have used alcohol or drugs in such a way that it has caused you worry	☐	☐	☐	☐

If you have experienced anything of the above, how much are you affected by that now?

☐ Not affected ☐ Affected to some extent ☐ Affected to a large extent

4. ILLNESS AND WORRIES

4.01 **Have you during the last month experienced any illness or injury?**

☐ Yes ☐ No

If YES: have you during the same period? (Tick once for each line)

	Yes	No
Been to a general practitioner	☐	☐
Been to a medical specialist	☐	☐
Been to emergency department	☐	☐
Been admitted to a hospital	☐	☐
Been to an alternative practitioner *(chiropractor, homeopath or similar)*	☐	☐

4.02 **Have you noticed sudden changes in your pulse or heart rythm in the last year?**

☐ Yes ☐ No

4.03 **Do you become breathless in the following situations?** (tick once for each question)

	Yes	No
When you walk rapidly on level ground or up a moderate slope	☐	☐
When you walk calmly on level ground	☐	☐
While you are washing or dressing	☐	☐
At rest	☐	☐

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

☐ Yes ☐ No

If YES: Is the cough usually productive?

☐ Yes ☐ No

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

☐ Yes ☐ No

4.05 **How often do you suffer from sleeplessness?** (tick once)

- ☐ Never, or just a few times a year
- ☐ 1-3 times a month
- ☐ Approximately once a week
- ☐ More than once a week

If you suffer from sleeplessness monthly or more often, what time of the year does it affect you most? (Put one or more ticks)

- ☐ No particular time
- ☐ Polar night time
- ☐ Midnight sun time
- ☐ Spring and autumn

4.06 **Have you had difficulty sleeping during the past couple of weeks?**

- ☐ Not at all
- ☐ No more than usual
- ☐ Rather more than usual
- ☐ Much more than usual

4.07 **Have you during the last two weeks felt unhappy and depressed?**

- ☐ Not at all
- ☐ No more than usual
- ☐ Rather more than usual
- ☐ Much more than usual

4.08 **Have you during the last two weeks felt unable to cope with your difficulties?**

- ☐ Not at all
- ☐ No more than usual
- ☐ Rather more than usual
- ☐ Much more than usual

4.09 **Below, please answer a few questions about your memory:** (tick once for each question)

	Yes	No
Do you think that your memory has declined?	☐	☐
Do you often forget where you have placed your things?	☐	☐
Do you have difficulties finding common words in a conversation?	☐	☐
Have you problems performing daily tasks you used to master?	☐	☐
Have you been examined for memory problems?	☐	☐

If YES to at least one of the first four questions above: Is this a problem in your daily life?

☐ Yes ☐ No

4.10 **Have you during the last last year suffered from pain and/or stiffness in muscles or joints in your neck/shoulders lasting for at <u>least 3 consecutive months</u>?**
(tick once for each line)

	No complaint	Little complaint	Severe complaint
Neck, shoulders	☐	☐	☐
Arms, hands	☐	☐	☐
Upper part of the back	☐	☐	☐
The lumbar region	☐	☐	☐
Hips, leg, feet	☐	☐	☐
Other places	☐	☐	☐

4.11 **Have you suffered from pain and/or stiffness in muscles or joints during the <u>last 4 weeks</u>?** (tick once for each line)

	No complaint	Little complaint	Severe complaint
Neck, shoulders	☐	☐	☐
Arms, hands	☐	☐	☐
Upper part of the back	☐	☐	☐
The lumbar region	☐	☐	☐
Hips, leg, feet	☐	☐	☐
Other places	☐	☐	☐

4.12 **Have you ever had:**

	Yes	No	Age last time
Fracture in the wrist/forearm?	☐	☐	☐☐
Hip fracture?	☐	☐	☐☐

4.13 **Have you been diagnosed with arthrosis by a physician?**
☐ Yes ☐ No

4.14 **Do you have or have you ever had some of the following:**

	Never	Some	Much
Nickel allergy	☐	☐	☐
Pollen allergy	☐	☐	☐
Other allergies	☐	☐	☐

4.15 **Have you ever experienced infertility for more than 1 year?**
☐ Yes ☐ No

If Yes: was it due to:

	Yes	No	Do not know
A condition concerning you?	☐	☐	☐
A condition concerning your partner?	☐	☐	☐

4.16 **To which degree have you had the following complaints during the last <u>12 months</u>?**

	Never	Some	Much
Nausea	☐	☐	☐
Heartburn/regurgitation	☐	☐	☐
Diarrhoea	☐	☐	☐
Constipation	☐	☐	☐
Alternating diarrhoea and constipation	☐	☐	☐
Bloated stomach	☐	☐	☐
Abdominal pain	☐	☐	☐

4.17 **If you have had abdominal pain or discomfort during the last year:**

	Yes	No
Was it located in your upper stomach?	☐	☐
Were you bothered as often as once a week or more during the last 3 months?	☐	☐
Do you feel symptoms relief after bowel movement?	☐	☐
Are the symptoms related to more frequent or rare bowel movements than normally?	☐	☐
Are the symptoms related to more loose or hard stool than normally?	☐	☐
Do the symptoms appear after a meal?	☐	☐

4.18 **Have you ever had:**

	Yes	No	Age last time
Gastric ulcer	☐	☐	☐☐
Duodenal ulcer	☐	☐	☐☐
Ulcer surgery	☐	☐	☐☐

4.19 **For women: Have you ever had a miscarriage?**
☐ Yes ☐ No ☐ Do not know
If Yes: number of times ☐☐

4.20 **For men: Have your partner ever had a miscarriage?**
☐ Yes ☐ No ☐ Do not know
If Yes: number of times ☐☐

4.21 **Is your diet gluten-free?**
☐ Yes ☐ No ☐ Do not know

4.22 **Have you been diagnosed with Dermatitis Herpetiformis (DH)?**
☐ Yes ☐ No ☐ Do not know

4.23 **Have you been diagnosed with coeliac disease, based on a biopsy from your intestine taken in a gastroscopy examination?**

☐ Yes ☐ No ☐ Do not know

4.24 **Do you have your natural teeth?**

☐ Yes ☐ No

4.25 **How many amalgam tooth fillings do you have/have you had?**

☐ 0 ☐ 1-5 ☐ 6-10 ☐ 10+

4.26 **Have you been suffering from headache <u>the last year</u>?**

☐ Yes ☐ No

If No: go to section 5, food habits

4.27 **What kind of headache are you suffering from?**

☐ Migraine ☐ Other headache

4.28 **How many days <u>per month</u> do you suffer from headache?**

☐ Less than one day
☐ 1-6 days
☐ 7-14 days
☐ More than 14 days

4.29 **Is the headache attacks <u>usually</u>:**
(tick once for each line)

	Yes	No
Pounding/pulsatory pain	☐	☐
Pressing/tightening pain	☐	☐
Unilateral pain *(right or left)*	☐	☐

4.30 **What is the normal intensity of your headache attacks?**

☐ Mild *(do not hinder normal activity)*
☐ Moderate *(decrease normal activity)*
☐ Strong *(block normal activity)*

4.31 **What is the normal duration of the headache attacks?**

☐ Less than 4 hours
☐ 4 hours – 1 day
☐ 1-3 days
☐ More than 3 days

4.32 **If you suffer from headache, when during the year does it affect you most?** (tick one or more)

☐ No particular time
☐ Polar night time
☐ Midnight sun time
☐ Spring and/or Autumn

4.33 **Before or during the headache, do you have a temporary:**

	Yes	No
Visual disturbances? *(flickering, blurred vision, flashes of light)*.....	☐	☐
Unilateral numbness in your face or hand?	☐	☐
Aggravated pain by moderate physical activity?	☐	☐
Nausea and/or vomiting?	☐	☐

4.34 **Describe how many days you have been away from work or school during the <u>last month</u> due to headache?**

Number of days..... ☐☐

5. FOOD HABITS

5.01 **How often do you usually eat the following?** (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
Fresh water fish *(not farmed)*	☐	☐	☐	☐
Salt water fish *(not farmed)*	☐	☐	☐	☐
Farmed fish *(salmon, trout, char)*	☐	☐	☐	☐
Tuna fish *(fresh or canned)*	☐	☐	☐	☐
Fish bread spread	☐	☐	☐	☐
Mussels, shells	☐	☐	☐	☐
The brown content in crabs	☐	☐	☐	☐
Whale or seal meat	☐	☐	☐	☐
Pluck (liver/kidney/heart) from reindeer or elk/moose	☐	☐	☐	☐
Pluck (liver/kidney/heart) from ptarmigan/grouse	☐	☐	☐	☐

5.02 **How many times during the year do/did you usually eat the following?** (number of times)

	In adulthood	In childhood
Mølje (cod or pollack meat, liver, and roe) *(Number of times per year)*	☐☐	☐☐
Sea gull's egg *(Number of eggs per year)*	☐☐	☐☐
Reindeer meat *(Number of times per year)*	☐☐	☐☐
Local mushroom and wild berries *(blueberries/lingonberries/cloudberries) (Number of times per year)*	☐☐☐	☐☐☐

5.03 **How many times per month do you eat canned (tinned) foods (from metal boxes)?**

Number ☐☐

5.04 **Do you take vitamins and/or mineral supplements?**

☐ Yes, daily ☐ Sometimes ☐ Never

5.05 **How often do you eat?**

	Never	1-3 times per month	1-3 times per week	4-6 times per week	1-2 times per day	3 times per day or more
Dark chocolate	☐	☐	☐	☐	☐	☐
Light chocolate/milk chocolate	☐	☐	☐	☐	☐	☐
Chocolate cake	☐	☐	☐	☐	☐	☐
Other sweets	☐	☐	☐	☐	☐	☐

5.06 **If you eat chocolate, how much do you usually eat each time?**

Compared with the size of a Kvikk-Lunsj sjokolade *(a chocolate brand in the market)* and describe how much do you eat in relation to it.

¼	½	1	1 ½	2	More than 2
☐	☐	☐	☐	☐	☐

5.07 **How often do you drink cocoa/hot chocolate?**

Never	1-3 times per month	1-3 times per week	4-6 times per week	1-2 times per day	3 times per day or more
☐	☐	☐	☐	☐	☐

6. ALCOHOL

6.01 How often have you in the last year:	Never	Less than monthly	Monthly	Weekly	Daily or almost daily
Not been able to stop drinking alcohol when you have started?	☐	☐	☐	☐	☐
Failed to do what was normally expected of you because of drinking?	☐	☐	☐	☐	☐
Needed a drink in the morning to get yourself going after a heavy drinking session?	☐	☐	☐	☐	☐
Had feeling of guilt or remorse after drinking?	☐	☐	☐	☐	☐
Not been unable to remember what happened the night before because of your drinking?	☐	☐	☐	☐	☐

	Never	Yes, but not in the last year	Yes, during the last year
6.02 **Have you or someone else been injured because of your drinking?**	☐	☐	☐
Has a relative, friend, physician, or other health care workers been concerned about your drinking or suggested you to cut down?	☐	☐	☐

7. WEIGHT

7.01 **Have you involuntary lost weight during the last 6 months?**

☐ Yes ☐ No

If Yes: how many kilograms? ..... ☐☐

7.02 **Estimate your body weight when you were 25 years old:**

Number of kilograms ..... ☐☐☐

7.03 **Are you satisfied with your present body weight?**

☐ Yes ☐ No

7.04 **What weight would you be satisfied with (your "ideal" weight)?**

Number of kilograms ..... ☐☐☐

8. SOLVENTS

8.01 **How many hours per week, do you do the following leisure- or professional activities:**

Automobile repair/paint, ceramic work, painting/varnishing/solvents, hair dressing, glazier, electrician. (Put 0 if you do not engage in such leisure or professional activities)

Number of hours per week on average ☐☐

8.02 **Do you use hair color preparations**

☐ Yes ☐ No

If Yes: How many times per year? .. ☐☐

9. USE OF HEALTH SERVICES

9.01 **Have you ever experienced that diseases have been insufficiently examined or treated, and this had a serious consequence?**

- ☐ Yes, this has happened to me
- ☐ Yes, this has happened to a close relative *(child, parents, spouse)*
- ☐ No

If Yes, was it caused by? (tick once or more):

- ☐ general practitioner
- ☐ emergency medical doctor
- ☐ private practising specialist
- ☐ hospital doctor
- ☐ other health personnel
- ☐ alternative practitioner
- ☐ more than one person due to deficient routines and interaction

9.02 **Have you ever felt persuaded to accept an examination or treatment that you did not want?**

☐ Yes ☐ No

If Yes, do you think this has had unfortunate consequences for your health?

☐ Yes ☐ No

9.03 **Have you ever complained about a treatment you have received?**

- ☐ Have never had a reason for complaining
- ☐ Have considered complaining, but did not do
- ☐ Have complained verbally
- ☐ Have complained in writing

9.04 **How long have you had your current general practitioner/other physician?**

- ☐ Less than 6 months
- ☐ 6 to 12 months
- ☐ 12 to 24 months
- ☐ More than 2 years

9.05 **At the last visit to your GP, did you have a hard time to understand what the doctor(s) told you?** Answer on a scale from 0 to 10, where 0 = they were difficult to understand and 10 = they were always easy to understand

0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 ☐

9.06 **How would you rate the treatment or counselling, you got at your last visit to your GP?** Answer on a scale from 0 to 10, where 0 = worst treatment or counselling, and 10 = best treatment or counselling

0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 ☐

9.07 **During the last 12 months, how much of a problem, if any, was it to get a referral to special examinations (as x-ray, etc.) or to a specialist health care (private practising specialist or at hospital)?**

- ☐ Not relevant
- ☐ No problem
- ☐ Some problem
- ☐ Major problem

9.08 **During the last 12 months, how much of a problem, if any, was it to get a referral to physiotherapist, chiropractor, etc.?**

- ☐ Not relevant
- ☐ No problem
- ☐ Some problem
- ☐ Major problem

9.09 **Altogether, how much of a problem, if any, was it to get a referral to specialist health care?**

- ☐ Not relevant
- ☐ Very difficult
- ☐ Some difficulties
- ☐ Easy
- ☐ Very easy

9.10 **During the last 12 months, have you been examined or treated by the specialist health care?**

☐ Yes ☐ No

If Yes, did you have a difficult time to understand what the doctor(s) told you? Answer on a scale from 0 to 10, where 0 = they were difficult to understand and 10 = they were always easy to understand

0 1 2 3 4 5 6 7 8 9 10

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

9.11 **How would you rate the treatment or counselling you got at your last visit to a specialist?** Answer on a scale from 0 to 10, where 0 = worst treatment or counselling, and 10 = best treatment or counselling

0 1 2 3 4 5 6 7 8 9 10

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

9.12 **Have you ever, <u>previous to the year 2002</u>, had an operation at a hospital or a specialist clinic?**

☐ Yes ☐ No

9.13 **Have you, during the <u>last 12 months</u>, used herbal or natural medicine?**

☐ Yes ☐ No

9.14 **Have you, during the <u>last 12 months</u>, used meditation, yoga, qi gong or thai chi as self-treatment?**

☐ Yes ☐ No

10. USE OF ANTIBIOTICS

10.01 **Have you used antibiotics during <u>the last 12 months</u>?** (all penicillin-like medicine in the form of tablets, syrups or injections)

☐ Yes ☐ No ☐ Do not remember

If YES: What did you get the treatment for?
Have you taken many antibiotic treatments, tick for each treatment.

	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6
• Urinary tract infection *(bladder infection, cystitis)*	☐	☐	☐	☐	☐	☐
• Respiratory tract infection *(ear, sinus, throat or lung infection, bronchitis)*	☐	☐	☐	☐	☐	☐
• Other	☐	☐	☐	☐	☐	☐
Treatment duration: number of days	|	|	|	|	|	|

How did you acquire the antibiotics for treatment?
Have you acquired many treatments, tick for each one.

With prescription from a physician/dentist	☐	☐	☐	☐	☐	☐
Without contacting a physician/without prescription:						
· Purchase from a pharmacy abroad	☐	☐	☐	☐	☐	☐
· Purchase over the internet	☐	☐	☐	☐	☐	☐
· Remnants from earlier treatment at home	☐	☐	☐	☐	☐	☐
· From family/friends	☐	☐	☐	☐	☐	☐
· Other ways	☐	☐	☐	☐	☐	☐

10.02 **Do you presently have antibiotics at home?**

☐ Yes ☐ No

If YES:is this after an agreement with your physician for treatment of chronic or frequently recurring disease?

☐ Yes ☐ No

If No: how did you acquire this antibiotic? (Multiple ticks are possible)

Purchased from a pharmacy abroad .... ☐
Purchased over the internet ..... ☐
Remnants from earlier treatment ..... ☐
From family/friends ..... ☐
Other ways ..... ☐

10.03 **Would you consider using antibiotics without consulting your physician?**

☐ Yes ☐ No

If YES: which conditions would you treat in such situation? (multiple ticks are possible)

Common cold ..... ☐
Cough ..... ☐
Bronchitis ..... ☐
Sore throat ..... ☐
Sinusitis ..... ☐
Fever ..... ☐
Influenza ..... ☐
Ear infection ..... ☐
Diarrhoea ..... ☐
Urinary tract infection ..... ☐
Other infections ..... ☐

11. YOUR CIRCADIAN RHYTHM

We will ask you some questions about your sleeping habits

11.01 **Have you worked in a shift work schedule during the last 3 months?**

☐ Yes ☐ No

11.02 **Number of days per week which you <u>cannot</u> freely choose when you sleep (e.g. work days)?**

0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Then I go to bed at ..... ☐☐:☐☐

I get ready to fall asleep at ..... ☐☐:☐☐

Number of minutes I need to fall asleep ..... ☐☐

I wake up at ..... ☐☐:☐☐

With help of: ☐ Alarm clock ☐ External stimulus *(noise, family members etc.)* ☐ By myself

Number of minutes I need to get up ..... ☐☐

11.03 **Number of days per week which you <u>can</u> freely choose when you sleep** (e.g. free days or holidays

0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Then I go to bed at ..... ☐☐:☐☐

I get ready to fall asleep at ..... ☐☐:☐☐

Number of minutes I need to fall asleep ..... ☐☐

I wake up at ..... ☐☐:☐☐

With help of: ☐ Alarm clock ☐ External stimulus *(noise, family members etc.)* ☐ By myself

Number of minutes I need to get up ..... ☐☐

12. SKIN AND DERMATOLOGY

12.01 **How often do you usually take a shower or a bath?** (tick once)

- ☐ 2 or more times daily
- ☐ 1 time daily
- ☐ 4-6 times per week
- ☐ 2-3 times per week
- ☐ Once a week
- ☐ Less than once a week

12.02 **How often do you usually wash your hands with soap daily?** (tick once)

- ☐ 0 times
- ☐ 1-5 times
- ☐ 6-10 times
- ☐ 11-20 times
- ☐ More than 20 times

12.03 **Have you ever taken any antibiotics (penicillin and penicillin-like medicines) because of a skin disease, for example infected eczema, acne, non-healing leg ulcers, recurrent abscess?**

☐ Yes ☐ No

If Yes: How many times in average per year did you take antibiotics during the period you were most affected (tick once)

☐ 1-2 ☐ 3-4 ☐ More than 4 times

12.04 **Have you or have you ever had the following skin disorders?** (tick once for each line)

	Yes	No
Psoriasis	☐	☐
Atopic eczema (children's eczema)	☐	☐
Recurrent hand eczema	☐	☐
Recurrent pimples/spots for several months	☐	☐
Leg or foot ulcer that did not heal for 3-4 weeks	☐	☐

If YES on the question concerning leg and/or foot ulcer, do you have any leg ulcer today?

☐ Yes ☐ No

12.05 **Have you often or always any of the following complaints?** (tick once for each line)

	Yes	No
Swelling in the ankles or legs, particularly in the evenings	☐	☐
Varicose veins	☐	☐
Eczema (red, itchy rash) on your legs	☐	☐
Leg pain that is getting worse when you are walking and is relieved when you are standing still	☐	☐

12.06 **Have you ever had the following diagnoses by a physician?** (tick once for each line)

	Yes	No
Psoriasis	☐	☐
Atopic eczema	☐	☐
Rosacea	☐	☐

12.07 **Have you recurring large acne/abscesses that are tender/painful and often form scars in the following places?** (tick once for each line)

	Yes	No
Armpits	☐	☐
Under the breasts	☐	☐
Stomach groove/the navel	☐	☐
Around the genitalia	☐	☐
Around the anus	☐	☐
The groin	☐	☐

If Yes: Have you ever visited a physician because of abscesses?

☐ Yes ☐ No

If Yes, did you get any of the following treatments? (tick once for each line)

	Yes	No
Antibiotic ointment	☐	☐
Antibiotic tablets	☐	☐
Surgical drainage	☐	☐
A larger surgical intervention including skin removal	☐	☐
Surgical laser treatment	☐	☐

Appendix 2

The SCL-10 questionnaire
Participants were asked to response to the following items according to their experience during the previous week
1. Suddenly scared for no reason 2. Feeling fearful 3. Faintness, dizziness, or weakness 4. Feeling tense or keyed up 5. Blaming yourself for things 6. Difficulty in falling asleep or staying asleep 7. Feeling blue 8. Feeling of worthlessness 9. Feeling everything is an effort 10. Feeling hopeless about future
Out of the 10 items described above, the first 4 items were related to anxiety and the remaining to depression. Each item was scored on a scale from 1 (not at all) to 4 (a lot)

Appendix 3

Beck Depression Inventory-II
Participants were asked to rate the following items according to their feelings during the past two weeks
1. Sadness
2. Pessimism
3. Past failure
4. Loss of pleasure
5. Guilty feelings
6. Punishment feelings
7. Self-dislike
8. Self-criticalness
9. Suicidal thoughts or whishes
10. Crying
11. Agitation
12. Loss of interest
13. Indecisiveness
14. Worthlessness
15. Loss of energy
16. Changes in sleeping pattern
17. Irritability
18. Changes in appetite
19. Concentration difficulty
20. Tiredness or fatigue
21. Loss of interest in sex
Each item was rated from 0 (no symptoms/change) to 3 (most symptoms/change) Adapted from *Beck, A., Steer, R., and Brown, G. The Beck Depression Inventory – Second Edition Manual. San Antonio, TX: The Psychological Corporation.1996*

Appendix 4

The Hospital Anxiety and Depression Scale

The participants were asked how they agree with the following statements

1. I feel tense or wound up
2. I get a sort of frightened feeling as if something bad is about to happen
3. Worrying thoughts go through my mind
4. I can sit at ease and feel relaxed
5. I get a sort of frightened feeling like butterflies in the stomach
6. I feel restless and have to be on the move
7. I get sudden feelings of panic
8. I still enjoy the things I used to enjoy
9. I can laugh and see the funny side of things
10. I feel cheerful
11. I feel as if I am slowed down
12. I have lost interest in my appearance
13. I look forward with enjoyment to things
14. I can enjoy a good book or radio or TV program

Question 1-7 is related to anxiety (HADS-A), and question 8-14 to depression (HADS-D)

The items were rated from 0-3, 0 being least depressed/anxious and 3 being the most.

Adapted from *Zigmond, A.S. and Snaith, R.P. The Hospital Anxiety and Depression Scale Acta Psychiatr Scand, 1983, 67: p. 361-370.*

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