UNIVERSITY OF TROMSØ UIT

Faculty of Health Sciences
Department of Community Medicine

The Development and Use of a New Tool for Estimating Individual Sun Induced Vitamin D in Epidemiological Surveys

Kåre Edvardsen

A dissertation for the degree of
Philosophiae Doctor

November 2010

The Development and Use of a New Tool for Estimating Individual Sun Induced Vitamin D in Epidemiological Surveys

By Kåre Edvardsen

Department of Community Medicine,
University of Tromsø, Norway

Table of contents

ACKNOWLEDGEMENTS

I want to express my gratitude to my supervisor, Magritt Brustad, and also Eiliv Lund, who gave me the opportunity to conduct this research for my doctoral dissertation. Your enthusiasm, encouragement and support have been extremely valuable.

I also want to thank my co-supervisor, Ola Engelsen, for his contribution to this work, with his excellent professional and linguistic skills.

Tonje Braaten, thank you for your patience giving me an insight into epidemiology.

Tom Wilsgaard, thank you for your contribution to this thesis. Your professional skills have been very helpful.

Many thanks to Lage Aksnes at the Haukeland University Hospital in Bergen and Bente Augdal at the Department of Community Medicine, University of Tromsø. Without your experience and effort when things became difficult, the first paper would not have been completed successfully.

Marit B. Veierød, I am most grateful for your contribution to the last paper. It has been a privilege working with you.

Thanks to the volunteers at Andøya Air Station, and to everyone else who took the time and effort to answer the questionnaires, and who gave blood samples for vitamin D analyses in the studies at Andenes.

Thank you to all the women who participated in the NOWAC-cohort. Without the support from you, this thesis could not have been accomplished.

The completion of this thesis would not have been possible without the financial support of Helse og Rehabilitering, The Norwegian Cancer society, The Norwegian Women and Cancer Study, and my employer, The Norwegian Institute for Air Research. Thank you!

Finally, I want to thank my family for being endlessly patient with me during the time it took to complete this work.

Tromsø, April 2010

Kåre Edvardsen

LIST OF PAPERS

1. Edvardsen K, Brustad M, Engelsen O, Aksnes L. The solar UV radiation level needed for cutaneous production of vitamin D-3 in the face. A study conducted among subjects living at a high latitude (68 degrees N). *Photochemical & Photobiological Sciences*. 2007;6(1):57-62.

2. Brustad M, Edvardsen K, Wilsgaard T, Engelsen O, Aksnes L, Lund E. Seasonality of UV-radiation and vitamin D status at 69 degrees north. *Photochemical & Photobiological Scienses.* 2007;6(8):903-908.

3. Edvardsen K, Engelsen O, Brustad M. Duration of vitamin D synthesis from weather model data for use in prospective epidemiological studies. *International Journal of Biometeorology.* 2009;53(5):451-459.

4. Edvardsen K, Veierød MB, Brustad M, Braaten T, Engelsen O, Lund E. Vitamin D-effective solar UV-radiation, dietary vitamin D and breast cancer risk. *International Journal of Cancer.* 2010; [Epub ahed of print].

LIST OF ABBREVIATIONS

UV-radiation: ultraviolet radiation.

UV-exposure: exposure by any UV-radiation

VD-radiation: vitamin D effective UV-radiation (integrated over the vitamin D-effective action spectrum).

VD-dose: VD-radiation integrated over time.

VD-hour: one hour duration of VD-radiation.

UV-hour: old notation of VD-hour, used in paper 1 and 2.

BED: biologically effective UV-dose, old notation for VD-dose, used in paper 1.

INTRODUCTION

When the human skin is sufficiently irradiated by vitamin D effective solar ultraviolet (UV) radiation (VD-radiation), vitamin D_3 (cholecalciferol) is formed through a photochemical reaction. This vitamin is essential in numerous biological functions, like the calcium and phosphate regulation. Now it is also well known that various organs in the body including the skin, colon, prostate and breast, are capable of synthesizing at need the active form of vitamin D, i.e. calcitriol(1-3). The interest in the vitamin has grown throughout the last 30 years as it was discovered to play a positive role in cell growth regulation, which is particularly of interest in cancer research. In vitro experiments showed in the early 1990's that vitamin D could have a dose-response related effect, by inhibiting cell growth of human colorectal cancer cells(4).

History of vitamin D and health

In the early 1820's Sniadecki,(5) noticed that children living in rural areas of Poland did not develop rickets. He believed the cause was that children living on farms were more exposed to sunlight than urban children. This hypothesis was later supported in the late 19th century by a British missionary and epidemiologist, Theodore Palm, as he believed that the incidence of rickets could be negatively associated with sunlight exposure,(6) when he noticed that children living in equatorial areas did not develop rickets.

The hypothesis of solar exposure as a cure for rickets suggested by Sniadecki and Palm was never really recognized and a cure for the decease was not discovered until Sir Edward Mellanby, through more than 100 experiments, found in 1918 that cod liver oil fed to rachitic dogs cured them from the decease within a few months(7). In the next few years a chemist at the University of Wisconsin, Elmer V. McCollum, investigated the chemical properties of cod liver oil further, and concluded through heating and oxidation of cod liver oil that there were at least two different active

compounds. The compound that oxidized and was not heat resistant, and the heat stable component, became known as vitamin A and vitamin D, respectively.

It was now known that solar exposure of human skin could prevent rickets, and this led to various experiments trying to isolate the precursor of vitamin D(8-11). By irradiating various foods with UV-radiation, it was found that only foods containing cholesterol had an anti rachitic effect, and in 1937 Adolf Windaus (Nobel prize winner in Chemistry of 1928) and his colleagues found the precursor of vitamin D_3 we know as 7-dehydrocholesterol(12).

A few years later, Apperly(13) observed that mortality rates from internal cancers were lower in hot climates compared to the cold climates over USA and Canada, and suggested that solar radiation could have a preventive effect on internal cancer mortality. But his conclusions were not related to UV-radiation induced vitamin D, and it was going to take another almost 40 years until vitamin D was associated with cancer prevention.

By studying the research literature from the last 30 years, or so, starting with the Garland's in 1980(14), there is no doubt that vitamin D has been a topic of great interest regarding prevention and treatment of various cancers and autoimmune deceases(15;16), but causality is yet to be proven(17).

Sources and metabolism of vitamin D

In order to increase the human blood-level of vitamin D you either have to expose your skin to VD-radiation for a sufficient amount of time(18) (VD-radiation integrated over time is defined as VD-dose) or increase the intake of vitamin D through diet or supplements. In general, the exposure to solar VD-radiation is the main source for vitamin D the year around, but that only applies to populations living in areas where the sun is high enough in the sky throughout the year in order to be strong enough to start the process of cutaneous production of vitamin D(19). Around 70° north (northern Norway) cutaneous production of vitamin D is absent from early October to the middle

March(20). We call that period the vitamin D winter, and around 60° north (Nordic countries, Canada, Alaska and Russia) the vitamin D winter is around 7 weeks shorter.

During this darker period of the year, the blood level of the vitamin will decrease, unless we take precautions. Intakes of fortified food, or supplements, or a diet with food naturally rich on vitamin D, like fatty fish and liver from fish, are needed for maintaining satisfactory vitamin D status. Although solar VD-radiation is the main source to vitamin D, both solar and artificial UV-radiation may positively be associated with a higher risk of melanoma(21). However, advice on sun avoidance is not straightforward, because supplements or carefully selected vitamin D food intake are then required(22).

Various habits regarding solar exposure and diet causes a great variation in the blood levels of vitamin D in the general population. The blood levels of vitamin D, increases with higher intake, following a dose-response function. However, in order to reach toxic levels, the intake has to be remarkably high(23), and is unreachable through a normal diet.

The process of cutaneous production of vitamin D has it's own down-regulation mechanism preventing intoxication. From cutaneous production in the epidermis to its active form in the bloodstream, the vitamin undergoes several stages. The epidermis is one of the human body's largest organ primarily built of keratinocytes, arranged in layers with the *stratum basale* as the bottom layer followed by *stratum spinosum, -granulosum, -lucidium* and *stratum corneum* as the outer layer.

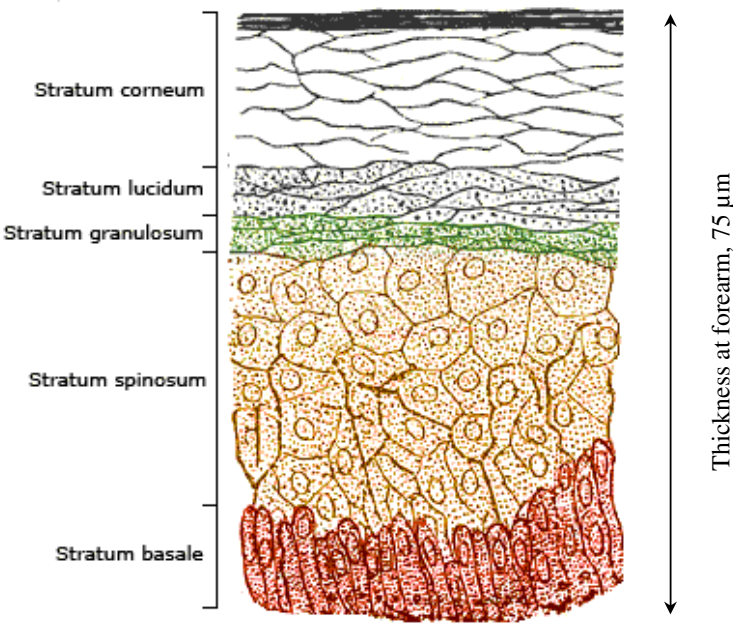


Figure 1. Epidermal strata of the human skin. Provitamin D_3 is found in highest concentrations in the *stratum basale* and the *stratum spinosum*.

(Source: http://en.wikipedia.org/wiki/File:Skinlayers.png)

In the first stage, 7-dehydrocholesterol (provitamin D_3) found in highest concentrations in the *stratum basale* and the *stratum spinosum*, is photochemically converted to previtamin D_3 through VD-radiation.

The concentration of provitamin D_3 is the first limiting factor of the cutaneous production of vitamin D_3, and is dependent of age. MacLaughlin and Holick(24) reported concentrations in epidermis of about 1 µg/cm^2 skin in younger people (< 20 yr) and about half the concentration in elderly people (> 70 yr), but later findings(25) suggest higher concentrations (above 2 µg/cm^2).

Only about 15% of the epidermal provitamin D_3 is converted to previtamin D_3 as the provitamin also is photochemically converted into mainly two other biologically inert products called lumisterol and tachysterol(26;27). Then, through a temperature dependent isomerization, which takes about 3 days at 37˚C, previtamin D_3 is converted to cholecalciferol (vitamin D_3), the form we

find in fatty fish, fish liver and supplements(27). At this stage the vitamin is transported to the blood by binding to the vitamin-D binding protein (DBP) present in the capillary bed of the dermis and enters the general circulatory system(26). As the photochemically process of converting provitamin D_3 into lumisterol and tachysterol is a much quicker process than the temperature dependent isomerization of provitamin D_3 and that provitamin D_3 comes from a finite source, intoxication of vitamin D_3 through exposure of the skin to VD-radiation is impossible.

As vitamin D_3 enters the blood stream it is still in a state that is biologically inactive with respect to calcium and phosphate regulation. The next step in the metabolic process is the hydroxylation in the liver to form 25-hydroxyvitamin D_3 (calcidiol), and the final 1-alpha-hydroxylase of calcidiol happens primarily in the kidney but also in other tissues of the body(1), providing 1,25-dihydroxyvitamin D_3 (calcitriol), known as the active vitamin D metabolite.

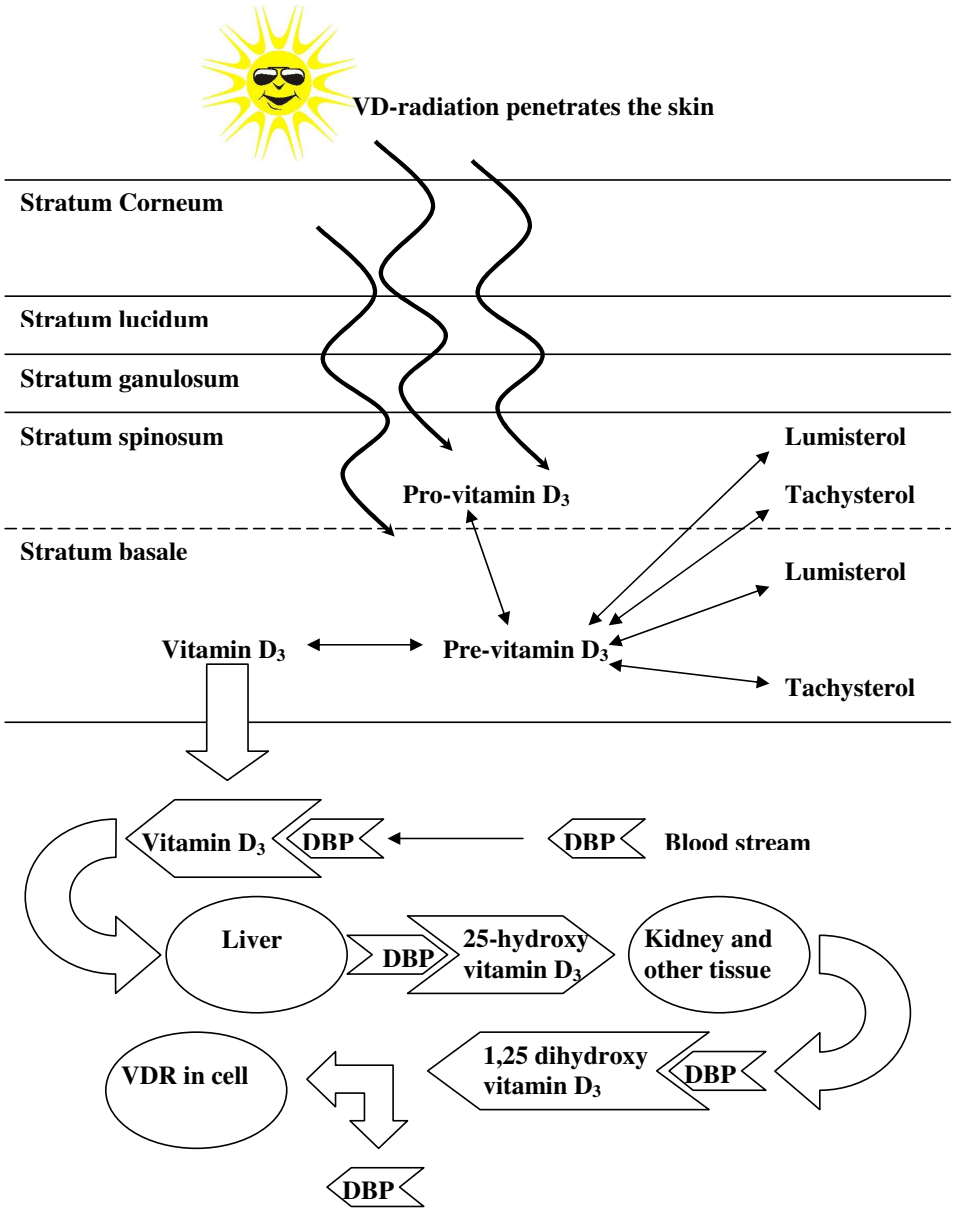


Figure 2. The vitamin D metabolism pathway from the skin to the destination cell. The transformation from pro-vitamin D_3 to pre-vitamin D_3 is restricted to stratum -spinosium and -basale. Through isomerization vitamin D_3 is formed and transported to the liver via the blood stream by the vitamin D binding protein (DBP). Then 25(OH)D_3 is formed through hydroxylation and further transported to the kidneys and other tissues for the final 1-alpha hydroxylase into 1,25 dihydroxy vitamin D_3, the active metabolite that binds to the vitamin D receptor in the destination cell.

Vitamin D proxies as exposures in epidemiology

Cancer of the breast is the most common cancer in women worldwide accounting for over 23 % of cancers in women (about 11% overall). Naturally, knowledge on how to prevent breast cancer, is appreciated(28). The main purpose in epidemiological studies is to estimate each exposure's effect on the outcome (i.e. death or diseases), and then it is important to include the most relevant types of exposures. Usually metrics extracted from questionnaires are used for this propose.

Estimating a subject's vitamin D status it can be done relatively easily and accurately by taking a blood sample and analyse it for vitamin D, but it is rather time consuming and expensive in an epidemiological study, usually including several thousand subjects. Since the vitamin can be obtained both orally and cutaneously, a way of estimating the status is to calculate vitamin dietary intake and how much vitamin D is produced in the skin. The usual way of estimating the oral contribution to the vitamin D status is to use questionnaires including questions on how much food and supplements a person is consuming on a regular basis and estimate the usual intake per day of the vitamin.

Estimating the contribution to the vitamin D status from the cutaneous production is challenging, as it depends on several factors. The main determinants to cutaneous vitamin D production are:

- The intensity of the VD-radiation
- Size of the skin area exposed by VD-radiation
- Duration of exposure
- Skin colour
- Age

Obviously, age and probably skin colour, are the easiest to collect information on in a large epidemiological study, although a person's skin colour is not an absolute value, as it varies mostly with melanin and thickness. To obtain extensive and accurate data on both the VD-radiation and

how much skin that was exposed by VD-radiation, in addition to the duration of the radiation, is far more difficult.

In epidemiology, a common way to select measures of exposure is to look for variables correlating with the outcome. As the VD-dose in general is stronger towards the equator, latitude has often been used as a proxy for vitamin D status in larger population-based studies(14;29;30). However, latitude only accounts for the latitudinal variation in the VD-dose, and not for variations caused by cloudiness, atmospheric ozone, and other variables affecting the VD-dose. Although VD-dose is a better proxy for vitamin D status than latitude, it is virtually absent in former epidemiological studies. Personal UV exposure assessments with vitamin D determination at an individual level have not been applied in epidemiological studies. Throughout 4 papers, forming the basis of this thesis, the development of a tool for generating and applying the VD-dose data in an epidemiological study, is presented.

AIMS OF THE THESIS

General aim

The general aim of this work has been to develop an improved methodological tool to estimate sun induced vitamin D at an individual level for use in epidemiological research. In addition, we implemented this tool in an assessment of the relationship between vitamin D status and breast cancer risk in the Norwegian Women and Cancer Study.

Specific aims

- To find the theoretical threshold value of VD-radiation for cutaneous production of vitamin D_3
- To find the time of year corresponding to the threshold value to verify if the sun is strong enough for cutaneous production of vitamin D_3 by assimilating measured VD-dose and vitamin D_3 (cholecalciferol) levels from human blood samples from northern Norway
- To investigate the seasonal variation of vitamin D status in relation to diet and outdoors habits in a North-Norwegian population
- To develop a tool for calculating historical VD-radiation at an arbitrary location, and produce this data for all of Norway
- To assess the significance of the vitamin D_3 status of women from the "Norwegian Women and Cancer Study" in relation to breast cancer by using information on intake of vitamin D_3 and exposure to VD-dose, at an individual level

MATERIALS AND METHODS

In the presented work, observational designs were used (table 1). In addition, model simulations were used to provide proxy data for the exposure variables in the cohort design. In the experimental designs the measurements of ambient VD-dose were used to assess the relation between VD-dose, the subjects' outdoors habits, and their vitamin D_3 status.

Table 1. Summary of the studies with respect to design, population, exposure, tools and measurements utilized.

Paper	Execution of work	Exposure	Tool	Outcome
1	Observational field study at Andenes, Northern-Norway, n=15	Weekly accumulated VD-dose General vitamin D intake	Time sheets (Appendix I) Questionnaire (Appendix I) blood sample	 25(OH)D blood concentrations
2	Prospective study through one year at Andenes, Northern-Norway, n=60	Weekly accumulated VD-hours General vitamin D intake	Time sheets (Appendix II) Questionnaire (Appendix II,III) blood sample	 25(OH)D blood concentrations
3	Development of a method for calculation of an exposure variable		VD-hours simulation tool	
4	Prospective cohort study, nationwide, n=41,811	20-year mean VD-dose, sun seeking holidays, solarium, sunburn General vitamin D intake at baseline	VD-dose calculation tool NOWAC questionnaire (Appendix III)	Breast cancer

Study populations

In the observational designs presented in paper 1 and 2, the study populations were volunteers from Andenes - a rural coastal site in northern Norway. The main reason for choosing Andenes was that continuous measurements of ambient UV-radiation were taken from a nearby observatory. At Andenes we also had a special interest in studying the effect of high dietary intake of vitamin D from coastal dietary traditions.

The cohort study (paper 4) was based on data from the Norwegian Women and Cancer Study (NOWAC). This cohort was chosen based on the common information on UV-exposure and dietary intake of vitamin D, for the subjects.

Blood sampling

In both studies described in paper 1 and 2, blood samples were drawn from the participants and analysed for the sum of vitamin D_3 and D_2. The procedures for blood sampling and methods for analysing for vitamin D in both studies are the same, and described in the individual papers. The Department of Paediatrics, Haukeland University Hospital, Bergen, Norway, was responsible for the actual laboratory analysis of the blood samples.

UV-radiation measurements

In all papers, except paper 4, solar UV spectral measurements were utilized. In paper 1, weekly integrated biologically effective UV dose rate (BED-rate) for photo-conversion of provitamin D to previtamin D, was compared with blood levels of vitamin D from each participant of the study, to assess the effect of their time spent outdoors in daylight, on their blood levels of vitamin D. This experiment was carried out over 10 consecutive weeks, except the week of Easter, during late winter of 2005. The participants were encouraged to spend at least 20 minutes outdoor every day. In

paper 2, exactly the same method was applied for assessing the UV-radiation, but instead of using the BED-rate value itself, the duration of the BED-rate (VD-hours) was measured the week prior to the blood sampling, and approximately every two months during one year, for examination of seasonal variation.

Real ambient UV-radiation was measured and the duration of radiation above an assumed threshold value for vitamin D production in skin was counted as VD-hours. The definition of BED-rate and one VD-hour, and how to apply them, is described in detail in paper 1 and 2, respectively. The instrument used for measurement of solar UV-radiation was a Brewer MK-III spectrophotometer manufactured by Kipp & Zonen, Delft, Netherlands (http://www.kippzonen.com). This instrument is especially designed to measure narrowband, spectral solar UV irradiance. The instrument is in continuous operation and is maintained according to a strict schedule, including relative and absolute calibration, ensuring high quality data. It is located at the ALOMAR observatory at Andøya Rocket Range, just outside the town of Andenes, where the subjects in the studies lived.

UV-radiation modelling

Model simulations can be of great value in lack of actual measurements, if they are within the required limits of accuracy. In paper 2, we have demonstrated the relation between time spent outdoors in VD-radiation conditions, and blood levels of vitamin D for 60 subjects from Andenes during one year. In this case the radiation conditions were measured by instruments on site, but the kind of instrumentation required is very limited and prevents studies of this kind to be carried out elsewhere, unless one can replace the measurements by model simulations of adequate accuracy.

In paper 3 we have demonstrated a method that can, to a certain extent, replace the UV-instruments based measurements with model simulations, for use in an epidemiological survey. The model simulations were compared with measurements over a wide range of conditions and found promising.

In paper 4 we have used model simulations of VD-radiation for all municipality centres in Norway during 1982-2002 as one of the main predictors of vitamin D status, in a large prospective epidemiological study.

All UV-radiation modelling is based on the libRadtran package, a library for radiative transfer calculation of solar and thermal radiation in the Earths atmosphere(31). The FastRT-model used in paper 3 and 4 is an offspring of the libRadtran package.

Statistics

Statistical analyses in paper 1, 3, and 4 were performed using the R software package, version 2.1.1 (The R foundation for Statistical Computing, Wien, Austria). In paper 2, SAS version 9.1 (SAS Institute, Cary NC, USA) was used. Various statistical issues are considered in detail in each paper. In general, linear regression was used in paper 1, 2 and 3. In paper 4, a Cox proportional hazards model was used. Both univariable and multivariable analyses were performed whenever appropriate.

Ethics

All three studies were approved by the Regional Ethical Committee, University of Tromsø, Norway. All participants in the two studies at Andenes (paper 1 and 2) had to complete a consent form. These studies were also reported to the Norwegian Data Inspectorate according to official regulations. The data used in paper 4 is from the NOWAC study, which has previously been approved for the collection and compilation of identifiable personal data.

SUMMARY OF RESULTS

Paper 1

The Solar UV radiation Level Needed for Cutaneous Production of Vitamin D_3 in the Face. A study Conducted Among Subjects Living at a High Latitude (69˚N).

Edvardsen K, Brustad M, Engelsen O, Aksnes L. *Photochemical & Photobiological Sciences* 2007; 6(1):57-62.

The purpose of the study was to determine the period during late winter for when the VD-radiation was strong enough to influence the blood level of vitamin D. A group of 15 people, 4 females and 15 males, at the age of 34 to 58 years participated in a study in the town of Andenes. The participants were asked to stay outdoors, exposing their face to daylight, around noon every day during the period of February 8 – April 12, 2005.

During the study period, 9 blood samples from each participant were analysed for vitamin D concentrations, ranging from 23.9 to 74.8 nmol/l between the subjects. Vitamin D from dietary intake showed that 7 subjects had lower intake than the recommended value of 7.5 µg/day. There were 5 of these subjects with vitamin D concentrations < 37.5 nmol/l, indicating a moderate hypovitaminosos D state.

No significant positive association between biologically effective UV-dose (BED) and vitamin D levels in blood for the group was found. A negative trend in mean vitamin D levels in blood was found for BED < ~7 kJ/m^2. For BED > ~7 kJ/m^2, there was a slight positive trend. Subjects with the lowest initial blood levels of vitamin D seemed to respond better to BED's than the subjects with the highest initial levels, for whom diet seemed to be the dominant factor.

Paper 2

Seasonality of UV-radiation and vitamin D status at 69 degrees north.

Brustad M, Edvardsen K, Wilsgaard T, Engelsen O, Aksnes L, Lund E. *Photochemical & Photobiological Sciences.* 2007;6(8):903-908.

The first paper provided valuable information of the impact of the BED on cutaneous vitamin D production on a relatively short term and when the VD-radiation and at the time of year when the VD-radiation was naturally week. The next step in the study was to assess seasonal variation in UV-radiation through one year and its impact on vitamin D status in subjects living at high latitude. The subjects, 44 females and 16 males, were between 20 and 60 years of age. Mean blood level of vitamin D for the study group was significantly highest in September and December (around 47 nmol/l) and was lowest in October and April (around 42 nmol/l). Exclusion of sun bed users and holidays travellers stabilized the vitamin D level around 40 nmol/l

In general, vitamin D status was inversely related to BMI, except for in June when no significant relationship was found. Subjects with BMI < 25, users of cod liver oil supplements, and those who consumed fish liver more than once per season, had a vitamin D level around 50 nmol/l.

The time each subject spent outdoors VD-radiation was estimated (VD-hours). For the study group, a significant, positive relationship was found at VD-hours ≥ 3.5. For subjects with at least one blood sample with a vitamin D level < 37.5 nmol/l, the positive relationship was found at VD-hours ≥ 1.5. For those who had all their levels above 37.5 nmol/l, the positive relationship was found at VD-hours ≥ 4.0.

Paper 3

Duration of vitamin D synthesis from weather model data for use in prospective epidemiological studies.

Edvardsen K, Engelsen O, Brustad M. *International Journal of Biometeorology.* 2009;53(5):451-459.

The knowledge from the previous papers on how time spent outdoors affected the blood levels of vitamin D for a group of people living at a high latitude formed the basis of this article, where we describe a method for calculation of historical datasets of weekly mean duration of VD-radiation (VD-hours), for an arbitrary location. Comparison between model results and measurements were done for Østerås in Oslo (60˚N) and at Andenes in Nordland (69˚N). All simulation and measurements were irradiance data weighted with the vitamin D effective action spectrum. In Oslo, the mean model-to-measurement ratio was 0.99 (SD 0.07) for solar zenith angles (SZA's) between 38˚ and 64˚. For SZA's > 64˚ the model-to-measurement ratio sometimes exceeded 50 %. At Andenes, the mean model-to-measurement ratio was 0.98 (SD 0.06) for SZA's between 46˚ and 61˚.

Trend analyses of the model results showed a significant negative trend of 13.2 min/decade ($p < 0.001$) during the period 1958 – 1977, and a significant positive trend of 8.4 min/decade ($p = 0.01$) during the period 1977 – 2001. The overall trend was small and insignificant.

Paper 4

Vitamin D and prevention of breast cancer. The prospective "Norwegian Women and Cancer Study"

Edvardsen K, Brustad M, Veierød MB, Braaten T, Engelsen O, Lund E. *International Journal of Cancer*; In review.

By using experience and achieved methodological knowledge obtained in the work described in paper 1 through 3, we constructed a Cox proportional hazards model from which we have examined the effects of dietary and solar induced vitamin D, on breast cancer, in a large cohort study (n = 41811, age 40 – 70). During 8.5 years of follow-up 948 cases of breast cancer were diagnosed.

Each subject in the study was assigned a mean potential vitamin D effective UV-dose (VD-dose), based on the adult lifetime places of residence. Mean VD-dose was 388.9 kJ/m^2/year (range 180.1 – 644.1 kJ/m^2/year). Mean vitamin D intake at baseline was 9.4 µg/day (range 0 – 67.3 µg/day). We did not find any strong relation between VD-dose and vitamin D intake, and the geographical distribution showed a slightly higher intake towards northern Norway.

No significant association was found between dietary vitamin D intake and breast cancer risk, neither in the age adjusted (P_{trend} = 0.96) nor multivariable analyses (P_{trend} = 0.69). We found a significant positive association between VD-dose and breast cancer risk in the age adjusted analyses (P_{trend} = 0.007), but no significant association was found in the multivariable analyses (P_{trend} = 0.21). Among the established risk factors, age (p <0.001), HT (current users, p <0.001), mother's history of breast cancer (p < 0.001), mammography (frequent, p = 0.02), and having 3 or more children after the age of 30 (p = 0.03) was significantly associated with breast cancer risk

GENERAL DISCUSSION

Introduction

The conclusion of the International Agency for Research on Cancer in their report from 2008 is that "The epidemiological evidence from observational studies suggests an inverse association between serum 25-hydroxyvitamin D levels and the incidence of breast cancer, but the differences between studies are large, and the overall evidence is weak when case-control studies are not included in the meta-analysis. New cohort studies on serum 25-hydroxyvitamin D levels and breast cancer risk are warranted"(17).

Some of the latest studies published suggest that vitamin D may be positively associated with reduced breast cancer risk(32-36), and other studies suggest that there is no association(37-39). Various proxies for VD-radiation have been used to assess the contribution of cutaneous vitamin D production to the vitamin D status of the subjects involved in these studies, and the different outcomes may raise a question about the validity of these measures.

In all studies, where a proxy variable is used to assess the vitamin D status, the proxy variable should have the highest possible correlation with the actual variable that would have influenced the variation in the vitamin D status. In general, there are three main factors that controls cutaneous vitamin D production: 1) the intensity of the VD-radiation, 2) the area of the skin which is exposed by VD-radiation, and 3) the duration of exposure, given the same skin colour. Obviously, in population based studies, point 2) and 3) is practically impossible to obtain. The essential question for us was: Could the intensity of the VD-radiation at the locations of interest be obtained within reasonable limits?

Previous work on reconstruction of historical solar UV-radiation data have been published for a location in Lofoten, northern Norway(40), and this led to the idea of finding a method of reconstructing the VD-radiation nationwide back to the late 50's, covering the adolescent lifetime for all subjects in the NOWAC cohort.

We saw this as a unique opportunity to take epidemiological studies involving vitamin D and breast cancer one step further, as we already had baseline information on vitamin D intake and activities related to solar exposure (information on holidays, sunburn, and use of solarium), and the lifetime history of places of residence.

By carefully examine the most relevant factors affecting the VD-dose, and its relation to the vitamin D status in a population, it was possible to develop a novel tool that could be used in an epidemiological study. The development was initiated with an experimental study (paper 1) on the relation on measured ambient VD-radiation and cutaneous vitamin D production, where the approximate limit for the required VD-radiation intensity was estimated. This unique information formed the basis for a prospective study (paper 2), where the yearly variation in blood levels of vitamin D from both VD-radiation induced, and dietary vitamin D, was examined. Through a methodological study (paper 3), based on the results of the two previous studies, a tool for producing historical VD-radiation data at any location was developed. In a final cohort study (paper 4), the tool has been used for the estimation of individual residential VD-dose data, and applied as a proxy for vitamin D status for each participant in the NOWAC study, in the assessment of a possible association between vitamin D status and risk of breast cancer.

The difference towards the north

Although, most people on a global scale live in areas with perpetually available cutaneous production of vitamin D, still around 6% (400 mill) of the Earth's population live above 51˚N (and below 51˚S) which is considered the geographical limit for possible cutaneous production of vitamin D year around(19;20). Going to the far north, more than 4 mill people live above the Arctic circle at 66˚N (no fixed population below 66˚S), where the vitamin D winter lasts for at least 4 months. Unless precaution is taken, unsatisfactory vitamin D status could be reached during this period(41). At least for the rural population, the solution for the lack of cutaneous vitamin D

production has been in the traditional marine diet like fatty fish, as salmon, trout and char, and fish liver which all are relatively rich on vitamin D. But also cod liver and cod liver oil played a key role in the diet(42). During 1928-1929, Johan Kloster carried out fieldwork on diet and rickets in the county of Finnmark, around 70˚ N in northern Norway(43). He discovered that the prevalence of rickets was strongly, inversely correlating with the access of fish, both due to season and location. He found that 50 % of the children under the age of 2, ether suffered from, or had clear signs of developing rickets, and in some communities up to 70% suffered from the decease during winter. Øgrim and Homb analysed data on dietary habits in Norway during 1947-1954, and found that vitamin D intake in general was too low before fortification of butter and margarine were introduced in 1950(44). In addition, vitamin D supplements were provided to pregnant women, infants over 6 weeks, and kids during the winter. Kloster's, and Øgrim and Homb's work did show that people living in sub arctic and arctic areas had to take precaution regarding intake of vitamin D, in particular during the winter when the cutaneous production of vitamin D was absent.

The general situation in Norway, with respect to vitamin D status in the population, has greatly improved since the 1950's due to the mentioned fortification strategy implementation. Still, subgroups of the population, and in particular some immigrants with a traditional diet low on vitamin D, have an unhealthy vitamin D status(45). This pattern can also be seen in other parts of the world, including Europe(46).

People living above 51˚N cannot solely rely on cutaneous vitamin D production from solar exposure. There are two ways of obtaining cutaneous vitamin D production during the vitamin D winter. One is using artificial UV-radiation sources (sun bed), and the other is to travel to sunny areas. Travelling on winter holidays to sunny places is common in Norway, but quite impractical and expensive for maintaining a healthy vitamin D status during the wintertime. The use of sun bed is more practical and less expensive, but both cases is associated with risk for cutaneous malignant

melanoma(21). From a public health point of view, maintaining a healthy vitamin D status through dietary intake is probably the easiest, less expensive, and safest option.

When is the vitamin D winter?

A person's vitamin D status is depending on the contribution of the cutaneous vitamin D production, and the amount of dietary vitamin D. These two variables may change over time and cause a change in the vitamin D status. In mid- and low-latitude areas, the yearly variation in VD-radiation is much less than in the sub arctic and arctic areas, and VD-radiation is also the main contributor to the vitamin D status in the populations living in the more sunny areas. This is not the case in sub arctic and arctic areas. Here, the sun is ether absent or too low in the sky for any cutaneous vitamin D production to occur for longer periods during the winter (about 3.5 months at 60˚, about 5 months at 70˚N).

In paper 1, we wanted to find the approximate time of year at Andenes (69˚N) when the ambient VD-radiation was strong enough to result in a measurable increase of vitamin D (25(OH)D) in blood in a study group of 15 people. Based on in vitro experiments on human skin by Webb et al.(47) in combination with radiative transfer (RT) modeling of VD-radiation, we estimated the level of VD-radiation to be just over 9 mW/m^2 (normalized to the vitamin D action spectrum), in order to have measurable effect. This value is normally reached around the 10^{th} of March, mostly depending on the cloud conditions and the thickness of the ozone layer. We decided to place this date in the middle of our test period of nine weeks, hoping that we could see both the decrease and increase in blood levels of 25(OH)D, as a result of insufficient and sufficient VD-radiation early and late in the test period, respectively.

Unfortunately, we failed to register any significant increase in 25(OH)D blood levels towards the end of the test period for the group, probably because of the very cloudy conditions at that time, preventing the VD-radiation to increase as much as needed. However, as we discovered that three

of the subjects, who had relatively low initial vitamin D status, seemed to somehow respond to the VD-radiation. Those with high initial vitamin D status did not seem to respond at all, except from dietary sources. These results were supported by the results from the study described in paper 2 (n = 60), where the group of people having an initial blood level of < 37.5 nmol/l, responded better to VD-radiation than the group of having an initial blood level of > 37.5 nmol/l.

The overall conclusion of the study in paper 1 was that we probably did find indications on when the vitamin D winter ends *in vivo*, but still that the diet was the dominant factor for the vitamin D status. If this study was to be re-designed, a methodological improvement by extending the study period by at least 4 more weeks, would have been preferred in order to obtain verifiable elevations in 25(OH)D values from higher VD-radiation exposure.

Seasonality of vitamin D status

As the major source of vitamin D for most humans is exposure to VD-radiation(48), a persons' vitamin D status most likely will have a yearly variation with the strength of the VD-radiation. The exception is when the dietary contribution to the vitamin D status is significant. We already knew that in Norway, the traditional diet is relatively rich in vitamin D, and we wanted to see the effect of the large change in VD-radiation in northern Norway, and diet, on the vitamin D status for a group of people consuming more traditional food. As described in paper 2, again, subjects from Andenes was selected. This is a fishery town and the population is likely to consume the very traditional dish called "mølje". This dish consists of potatoes, fresh cod, cod roe, and liver, which is very rich on vitamin D.

At two months intervals, throughout one year, the subjects recorded the time spent out doors in daylight (VD-hours) the last week before giving a blood sample for determination of blood levels of 25(OH)D, together with a questionnaire on dietary intake. Indeed, a low level of vitamin D status for the group was found in April (42.0 nmol/l) and a high level was found in September (46.7

nmol/), but a similar low and high level was found in October and December, respectively (Fig. 3). After adjusting for sex, age, BMI, vitamin D intake, solarium, and sun vacation in our statistical analyses, we found a significant association between the number of VD-hours and blood level of 25(OH)D for the study group, but also the dietary intake clearly influenced the level of 25(OH)D, and in particular during the period from December to March, when we observed an increased consumption of cod-liver oil and cod liver.

This survey clearly showed that the dietary contribution to vitamin D levels in blood neutralised the contribution of seasonal variation in sun exposure, but also that the VD-hours variable (named UV-hours in paper 2) could be a useful and valid exposure variable in an epidemiological context.

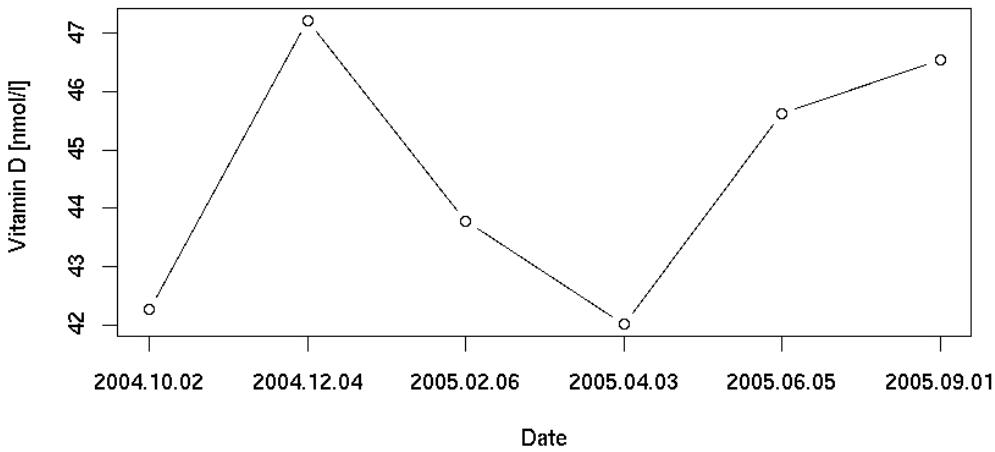


Figure 3. The yearly variation in mean blood levels of vitamin D for the subjects in the second Andens study (n = 60). The generally high dietary intakes of vitamin D, largely masks the effect of the seasonal variation in UV-exposure.

From VD-hour to VD-dose

In all calculations involving VD-radiation we have used the action spectrum for cutaneous vitamin D production as standardized by Commission Internationale de l'Eclariage (CIE)(18). VD-dose is defined as temporal integrated VD-radiation. One VD-hour is defined as one hour of VD-radiation. In paper 2, we demonstrated that the VD-hours could be used as a predictor for vitamin D for a group of people living at approximately the same latitudes. This might not be the case if the study group is spread over a wider range of latitude, as lower latitudes in general give stronger UV-radiation. Thus, the effectiveness of a VD-hour increases with decreasing latitude. One VD-hour around noon in Tromsø with clear sky the first of June corresponds to a VD-dose of approximately 490 J/m^2. The same value for Trondheim and Oslo is 660 J/m^2 and 770 J/m^2, respectively. In calculation of the VD-dose, the latitudinal effect is accounted for as the calculations include the effect of the SZA. If the latitudinal effect is not accounted for in a study, the exposure used (VD-hours), might lead to differential misclassification of the subjects (being misclassified in relation to their true exposure status).

The original reason for using VD-hours in favour of VD-dose was a combination of two matters. Firstly, in paper 2, we had only the information on how long, and not when the subjects had been outdoors, so estimation of the corresponding VD-dose was impossible. Secondly, all subjects reported time spent outdoors practically from the same latitude. Hence, the latitudinal effect was not an issue. Obviously, when the subjects in a study are recruited over a large range of latitudes, the latitudinal effect should be accounted for.

In preparation of data for paper 4, we knew that we would get misclassification of the subjects if we assumed equality between one VD-hour over the whole latitude range of Norway. By applying a latitudinal dependent weighting function to the VD-hours data, the problem of misclassification could probably be reduced, which would imply a recalculation of the VD-hours data. An estimation of time consumption for calculating a latitudinal dependent weighting function and applying it on

the VD-hours data, compared to calculating the VD-dose directly was not that different. In addition, we had got access to a high performance computational service (NOTUR – The Norwegian Metacenter for Computational Science, http://www.notur.no), thus, the decision for calculating the VD-dose for use in the final paper was made.

Methodological considerations and validity

In epidemiology, there are two basic types of study design, classified as either intervention (experimental) studies or observational studies. Experimental studies are characterized in the way that the investigator is controlling the exposure of the subjects, and noting the outcome of interest. In observational studies the role of the investigator is merely to observe who is naturally exposed or unexposed and noting the outcome of interest, without controlling the exposure.

The study design described in the first paper may be considered experimental, as the exposure was to some extent controlled. In this case, the exposure was personal VD-hours accumulated over a weekly basis, and the subjects were encouraged to go outdoors for a minimum of 20 minutes every day. Disregarding this small encouragement, the study designs in paper 1, 2, and 4, are well within the definition of observational study designs.

Paper 3 describes a method for calculating a variable for use in epidemiological studies and is fully based on a methodological development work. When conducting an epidemiological study, the main objective is to obtain the best valid and precise estimate on the effect of an exposure on a disease, or outcome, on the source population. The validity of a study, and to which degree the findings in a study can be generalized to others than the source population, is commonly referred to as internal and external validity (generalizability), respectively. In order to obtain a high validity, it requires that the variable used to describe an exposure, is estimated with little error. Error in estimates may be systematic or random. Systematic errors are commonly referred to as biases, and

the smaller the systematic error is, the more valid the study is. In general, biases are categorized as confounding, selection bias, and information bias.

Confounding

For a variable to be a confounder, it must be associated with the exposure under study, and simultaneously be an independent risk factor for the disease or outcome. Potential confounders in population-based studies will always exist, and it is essential for the validity of a study to keep the confounding effects at a minimum. This is only possible if the important confounders are identified and appropriately measured.

In paper 1 and 2, we wanted to see if the time spent outdoors, measured as VD-hours, could be associated with blood levels of vitamin D in our subjects. In both analyses, we adjusted for known confounders that could significantly influence the results (age, sex, BMI, sun seeking holidays, and use of solarium). Another possible confounder we did not account for that could have influenced the results was the area of exposed skin, which is often associated with the effective outdoors temperature, as it could have influenced how much the participants did cover up their skin, hence, reducing the cutaneous vitamin D production. To minimize the confounding effect, the subjects were asked to expose fairly the same area of skin throughout the study described in paper 1.

In the study described in paper 4, we have used data from the NOWAC questionnaire on vitamin D intake and activities related to VD-exposure, in order to estimate a vitamin D status for the subjects in the study. The main exposure was the last 20 years mean residential VD-dose together with sun seeking holidays, use of solarium and frequency of sunburns, and daily vitamin D intake, acting as proxies for vitamin D status. People living in areas with less sun may tend to seek to sunnier areas during holidays or use solarium, and without accounting for the most important habits related to cutaneous vitamin D production in our study, it could have negatively affected the validity.

Selection bias

When there is a systematic difference in the relevant characteristics of a population selected for a study compared to the population the selected participants are supposed to represent, it will lead to selection bias. This affects the external validity of a study, but not necessarily the internal validity

A common source of selection bias is self-selection, which is relevant to studies where the participants are volunteers. The studies described in paper 1 and 2, where based on volunteers, and could potentially have affected the external validity. However, the main purpose of the studies was to assess the variation in blood levels of vitamin D, in relation to variation in VD-exposure and diet, and there is no reason to believe that volunteers respond different to VD-exposure or diet, than the general population, and selection bias was hardly a concern.

Low response rates in population based studies have always been a concern, with respect to selection bias. The cohort study described in paper 4, was based on subjects who twice completed a NOWAC-questionnaire. The first time was during the years 1991 – 1997, with a crude response rate of 57%. The second time was during the years from 1998 – 2002, with a crude response rate of 81%. Thus, the final response rate was 46%. Similar response rates are found in other cohort studies on vitamin D and breast cancer risk(36;38;49;50).

After the first round of NOWAC questionnaires were returned, the distribution of exposure variables were compared in samples from the cohort, with various response rates (55% – 70%), and no statistically significant differences were found(51;52). However, it was found that a larger proportion of the responders had a higher socio-economic status (SES) measured as level of education, which previously has been associated with an increased risk of breast cancer. Braaten et al.(53) showed that the association could be fully explained by known risk factors, which estimates were found to be independent of SES, hence the results are probably not affected by selection bias. Also, when comparing the cumulated age-specific breast cancer incidence rates in NOWAC with

national figures(54), the numbers are almost identical, supporting a good external validity of the study described in paper 4.

When it comes to intake of vitamin D and outdoors habits in relation to VD-radiation, a potential source of selection bias could be the healthy volunteer effect(51). More physically active people in the NOWAC cohort have a significantly higher intake of vitamin D (results not shown). One might also think they tend to stay more outdoors exercising, hence, be more exposed to VD-radiation, compared to less physically active people. But using physical activity to describe the relationship between residential VD-radiation and true exposure to VD-radiation is probably not valid, as physical activity alone is by no means the only variable related to outdoors habits in solar VD-radiation conditions. The only way to map people's outdoors habits through questionnaires, in order to establish a measure on vitamin D status, is to ask where and when they were outdoors. Obtaining such information requires completion of exact time sheets, and such high levels of compliance cannot be expected for large cohort studies.

Information bias

When the means for obtaining information about the subjects in a study, regarding exposures, are inadequate, the collected information may be incorrect. This may lead to what is referred to as information bias in a study.

Misclassification is referred to as differential and nondifferential. In the case of differential misclassification, the association of an exposure and disease can bias the estimates in either direction, leading to an apparent association that really doesn't exist, or to miss an association that really exist. In the case of nondifferential misclassification, all subjects have the same probability of being misclassified in relation to their exposure status or outcome, thus diluting an eventual effect of an exposure.

The potential of differential misclassification is always an issue when it comes to questions that can be associated with life style. In general, when the subjects have knowledge on their outcome status, or the effects of the exposure variables, they tend to report differently. Classical examples are that people tend to under estimate their alcohol consumption, or that obese subjects tend to underestimate how much they eat.

In the papers 1 and 2, the subjects have answered a questionnaire with focus on diet rich on vitamin D, and time spent outdoors. In addition they gave blood samples frequently through the studies. The objective of the studies was to assess the effect of diet and outdoors habits on their vitamin D status. None of the subjects knew their vitamin D status during the study, reducing the risk for misclassification, as the subjects were not affected by knowledge of the outcome. Obviously, there was a potential of differential misclassification, as some subjects could have reported a healthier diet with more vitamin D, or reporting more time spent outdoors than actual, in order to appear more healthy. This could have lead to a weakening of the association between intake of vitamin D, outdoors habits, and vitamin D status.

Over- and underestimation of variables associated with a healthy or unhealthy lifestyle, respectively, is a well-known problem in cohort studies and indeed an issue for the NOWAC cohort. In paper 4, two of the main exposures in the analyses were VD-dose, based on self reported information on lifetime places of residence, and dietary vitamin D intake calculated from a food frequency questionnaire. We have no reason to believe that people systematically would report erroneously about lifetime places of residence, but there could be a possibility that subjects tend to report a healthier lifestyle with respect to diet, as discussed above, regarding paper 1 and 2. Thus, information bias is more likely in the NOWAC food frequency questionnaire in paper 4, hence, its validity has been described in detail earlier(55;56).

CONCLUDING REMARKS

Throughout the four papers presented in this thesis, a method development for estimating human vitamin D status in a large female cohort has been described, and the results when implementing this method on a large cohort study on vitamin D and breast cancer risk has been presented. The main conclusions may be summarized as follows:

- The vitamin D winter in Norway varies from 3.5 months in the south, to more than 5 months in the north. In general, the cutaneous vitamin D production is absent whenever the sun is lower than about 16˚ above the horizon, assuming an ozone layer thickness of ~300 DU.

- Subjects with low initial blood levels of vitamin D (< ~30 nmol/L), responds more effectively to VD-radiation than subjects with higher initial blood levels (> ~50 nmol/L).

- The vitamin D status for people living at high latitudes (Northern-Norway) is determined by the sum of dietary and cutanously obtained vitamin D. The dietary contribution to the vitamin D levels in blood partly neutralises the contribution of seasonal variation in VD-dose exposure.

- The "VD-hours" variable has been a useful epidemiological tool in combination with questionnaire data on vitamin D intake for predicting vitamin D status in a population living at high latitudes.

- Based on historical records on two main UV-radiation forcing factors, clouds and ozone, a record of daily VD-dose from 1957 – 2002, has been reconstructed with accuracy within reasonable limits.

- We found a significant positive association between VD-dose and breast cancer risk in the age-adjusted analysis, but no association was found when adjustment for known risk factors were done, suggesting that the known risk factors play a more important role than vitamin D in breast cancer etiology.

- No reduced risk of breast cancer among women who had lived in areas with high VD-dose as compared to those with low VD-dose, nor among women with high vitamin D intake compared to those with low intake was found, when adjusting for known risk factors. The results suggest that there is no association between vitamin D status and risk of breast cancer for women living at high latitudes.

FURTHER PERSPECTIVES

During the work that resulted in this thesis, several questions regarding vitamin D, both technically and health-related, was illuminated. In addition several new questions were raised, and are indeed subject to further investigations:

- The annual variation in vitamin D status for the Norwegian population is not very well known and needs to be further investigated. Not only for the population as a whole, but also with respect to geographical and dietary differences in Norway.

- The VD-dose data should be applied in other studies related to vitamin D and health, like colon cancer, and multiple sclerosis.

- The tool should be further developed to include other biologically effective doses beyond vitamin D, e.g. melatonin suppression and eye damage (cataracts).

- The reason for a lower breast cancer incidence rate towards the north of Norway is not well understood, and needs to be further investigated.

REFERENCES

(1) Zehnder D, Bland R, Williams MC, McNinch RW, Howie AJ, Stewart PM et al. Extrarenal expression of 25-hydroxyvitamin D-3-1 alpha-hydroxylase. Journal of Clinical Endocrinology and Metabolism 2001; 86(2):888-894.

(2) Holick MF. Vitamin D: A millenium perspective. J Cell Biochem 2003; 88(2):296-307.

(3) Townsend K, Banwell CM, Guy M, Colston K, Mansi JL, Stewart PM et al. Autocrine metabolism of vitamin D in normal and malignant breast tissue. Clinical Cancer Research 2005; 11(9):3579-3586.

(4) Thomas MG, Tebbutt S, Williamson RCN. Vitamin-D and Its Metabolites Inhibit Cell-Proliferation in Human Rectal Mucosa and A Colon Cancer Cell-Line. Gut 1992; 33(12):1660-1663.

(5) Sniadecki J. (Cited by Mozolowski, J.) On the cure of rickets. Nature 1939; 143:121.

(6) Palm T. The geographical distribution and etiology of rickets. The Practioner 1890; 45:270-279.

(7) Mellanby E. The part played by an 'accessory factor' in the production of experimental rickets. J Physiol 1918; 52:11-14.

(8) McCollum EV, Simonds N, Becker JE, Shipley PG. Studies on experimental rickets. XXI. An experimental demonstration of the existence of a vitamin which promotes calcium deposition. J Biol Chem 1922; 53:293-312.

(9) Goldblatt H, Soames KM. A study on rats on a normal diet irradiated daily by the mercury vapour quarts lamp or kept in darkness. Biochem J 1923; 17:294-297.

(10) Goldblatt H. A study of the relation of quantity of fat-soluble organic factor in the diet to the degree of calcification of the bones and the experimental development of rickets in rats. Biochem J 1924; 17:298-326.

(11) Steenbock H, Black A. Fat-soluble vitamins: XVII. The induction of growth-promoting and clarifying properties in a ration by exposure to ultra-violet light. J Biol Chem 1924; 61:405-422.

(12) Windaus A, Schenk F, Werder FV. Über das antirachitishi wirksame Bestrahlungsprodukt aus 7-Dehydrocholesterol. Z Physiol Chem 1936; 241:100-103.

(13) Apperly FL. The relation of solar radiation to cancer mortality in North America. Cancer Res 1941; 1:191-195.

(14) Garland CF, Garland FC. Do Sunlight and Vitamin-D Reduce the Likelihood of Colon Cancer? International Journal of Epidemiology 1980; 9(3):227-231.

(15) Mohr SB. A Brief History of Vitamin D and Cancer Prevention. Annals of Epidemiology 2009; 19(2):79-83.

(16) Holick MF. Vitamin D: a D-Lightful health perspective. Nutrition Reviews 2008; 66(10):S182-S194.

(17) Vitamin D and cancer. 5. 24-11-2008. Lyon, France, International Agency for Research on Cancer. IARC Working Group Reports ; 5.

Ref Type: Report

(18) Holick MF, Bouillon R, Eisman J, Garabedian M, Kleinschmidt J, Suda T et al. Technical report: Action spectrum for the production of previtamin D3 in human skin. CIE 174. 2006.

Ref Type: Report

(19) Edvardsen K, Brustad M, Engelsen O, Aksnes L. The solar UV radiation level needed for cutaneous production of vitamin D-3 in the face. A study conducted among subjects living at a high latitude (68 degrees N). Photochemical & Photobiological Sciences 2007; 6(1):57-62.

(20) Engelsen O, Brustad M, Aksnes L, Lund E. Daily duration of vitamin D synthesis in human skin with relation to latitude, total ozone, altitude, ground cover, aerosols and cloud thickness. Photochemistry and Photobiology 2005; 81(6):1287-1290.

(21) Veierod MB, Weiderpass E, Thorn M, Hansson J, Lund E, Armstrong B et al. A prospective study of pigmentation, sun exposure, and risk of cutaneous malignant melanoma in women. J Natl Cancer Inst 2003; 95(20):1530-1538.

(22) Vieth R. What is the optimal vitamin D status for health? Progress in Biophysics & Molecular Biology 2006; 92(1):26-32.

(23) Pettifor JM, Bikle DD, Cavaleros M, Zachen D, Kamdar MC, Ross FP. Serum Levels of Free 1,25-Dihydroxyvitamin-D in Vitamin-D Toxicity. Annals of Internal Medicine 1995; 122(7):511-513.

(24) Maclaughlin J, Holick MF. Aging Decreases the Capacity of Human-Skin to Produce Vitamin-D3. Journal of Clinical Investigation 1985; 76(4):1536-1538.

(25) Obi-Tabot ET, Tian XQ, Chen TC, Holick MF. A human skin equivalent model that mimics the photoproduction of vitamin D-3 in human skin. In Vitro Cellular & Developmental Biology-Animal 2000; 36(3):201-204.

(26) Norman AW. Sunlight, season, skin pigmentation, vitamin D, and 25-hydroxyvitamin D: integral components of the vitamin D endocrine system. American Journal of Clinical Nutrition 1998; 67(6):1108-1110.

(27) Holick MF. The Cutaneous Photosynthesis of Pre-Vitamin D3 - A Unique Photo-Endocrine System. Journal of Investigative Dermatology 1981; 77(1):51-58.

(28) Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. 2009. American Institute for Cancer Research.

Ref Type: Report

(29) Grant WB. An ecologic study of dietary and solar ultraviolet-B links to breast carcinoma mortality rates. Cancer 2002; 94(1):272-281.

(30) Grant WB. The likely role of vitamin D from solar ultraviolet-B irradiance in increasing cancer survival. Anticancer Research 2006; 26(4A):2605-2614.

(31) Mayer B, Kylling A. Technical note: The libRadtran software package for radiative transfer calculations - description and examples of use. Atmospheric Chemistry and Physics 2005; 5:1855-1877.

(32) Abbas S, Linseisen J, Slanger T, Kropp S, Mutschelknauss EJ, Flesch-Janys D et al. Serum 25-hydroxyvitamin D and risk of post-menopausal breast cancer - results of a large case-control study. Carcinogenesis 2008; 29(1):93-99.

(33) Blackmore KM, Lesosky M, Barnett H, Raboud JM, Vieth R, Knight JA. Vitamin D from Dietary Intake and Sunlight Exposure and the Risk of Hormone-Receptor-Defined Breast Cancer. American Journal of Epidemiology 2008; 168(8):915-924.

(34) Knight JA, Lesosky M, Barnett H, Raboud JM, Vieth R. Vitamin D and reduced risk of breast cancer: A population-based case-control study. Cancer Epidemiology Biomarkers & Prevention 2007; 16(3):422-429.

(35) Mohr SB, Garland CF, Gorham ED, Grant WB, Garland FC. Relationship between low ultraviolet B irradiance and higher breast cancer risk in 107 countries. Breast Journal 2008; 14(3):255-260.

(36) Robien K, Cutler GJ, Lazovich D. Vitamin D intake and breast cancer risk in postmenopausal women: the Iowa Women's Health Study. Cancer Causes & Control 2007; 18(7):775-782.

(37) Freedman DM, Chang SC, Falk RT, Purdue MP, Huang WY, McCarty CA et al. Serum levels of vitamin D metabolites and breast cancer risk in the prostate, lung, colorectal, and ovarian cancer screening trial. Cancer Epidemiology Biomarkers & Prevention 2008; 17(4):889-894.

(38) Kuper H, Yang L, Sandin S, Lof M, Adami HO, Weiderpass E. Prospective Study of Solar Exposure, Dietary Vitamin D Intake, and Risk of Breast Cancer among Middle-aged Women. Cancer Epidemiology Biomarkers & Prevention 2009; 18(9):2558-2561.

(39) McCullough ML, Stevens VL, Patel R, Jacobs EJ, Bain EB, Horst RL et al. Serum 25-hydroxyvitamin D concentrations and postmenopausal breast cancer risk: a nested case control study in the Cancer Prevention Study-II Nutrition Cohort. Breast Cancer Research 2009; 11(4).

(40) Engelsen O, Hansen GH, Svenoe T. Long-term (1936-2003) ultraviolet and photosynthetically active radiation doses at a north Norwegian location in spring on the basis of total ozone and cloud cover. Geophysical Research Letters 2004; 31(12).

(41) Webb AR, Engelsen O. Calculated ultraviolet exposure levels for a healthy vitamin D status. Photochem Photobiol 2006; 82(6):1697-1703.

(42) Brustad M, Sandanger T, Andersen V, Lund E. Consumption of fish-liver and risk of cancer - an epidemiological approach to the arctic dilemma. Epidemiology 2006; 17(6):S304-S305.

(43) Kloster J. The distribution and frequency of rickets in one of the fishery districts of Finnmark and relation of diet to the disorder. Acta Pædiatr Scand 1931; 12:1-82.

(44) Øgrim ME, Homb E. Kostvaner og næringstilførsel. Oslo: Universitetsforlaget, 1960.

(45) Madar AA, Stene LC, Meyer HE. Vitamin D status among immigrant mothers from Pakistan, Turkey and Somalia and their infants attending child health clinics in Norway. Br J Nutr 2009; 101(7):1052-1058.

(46) Lips P. Vitamin D status and nutrition in Europe and Asia. J Steroid Biochem Mol Biol 2007; 103(3-5):620-625.

(47) Webb AR, Kline L, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. J Clin Endocrinol Metab 1988; 67(2):373-378.

(48) Holick MF. Environmental-Factors That Influence the Cutaneous Production of Vitamin-D. American Journal of Clinical Nutrition 1995; 61(3):S638-S645.

(49) van den Brandt PA, Goldbohm RA, van ', V, Volovics A, Hermus RJ, Sturmans F. A large-scale prospective cohort study on diet and cancer in The Netherlands. J Clin Epidemiol 1990; 43(3):285-295.

(50) Manjer J, Carlsson S, Elmstahl S, Gullberg B, Janzon L, Lindstrom M et al. The Malmo Diet and Cancer Study: representativity, cancer incidence and mortality in participants and non-participants. Eur J Cancer Prev 2001; 10(6):489-499.

(51) Lund E, Kumle M, Braaten T, Hjartaker A, Bakken K, Eggen E et al. External validity in a population-based national prospective study--the Norwegian Women and Cancer Study (NOWAC). Cancer Causes Control 2003; 14(10):1001-1008.

(52) Lund E, Gram TI. The impact of design on response rates and exposure estimates from postal questionnaires. A population based randomized trial of 5000 Norwegian women aged 35-49 years. Scan J Soc Med 1998; 26:154-160.

(53) Braaten T, Weiderpass E, Kumle M, Adami HO, Lund E. Education and risk of breast cancer in the Norwegian-Swedish women's lifestyle and health cohort study. Int J Cancer 2004; 110(4):579-583.

(54) Cancer in Norway 1999. 2002. Kreftregisteret, Oslo, The Cancer Registry of Norway. Ref Type: Report

(55) Hjartaker A, Lund E, Bjerve KS. Serum phospholipid fatty acid composition and habitual intake of marine foods registered by a semi-quantitative food frequency questionnaire. Eur J Clin Nutr 1997; 51(11):736-742.

(56) Hjartaker A, Andersen LF, Lund E. Comparison of diet measures from a food-frequency questionnaire with measures from repeated 24-hour dietary recalls. The Norwegian Women and Cancer Study. Public Health Nutr 2007; 10(10):1094-1103.

ERRATA

Paper 1

The latitude in the title of the paper should be 69˚N.

Paper I

Paper II

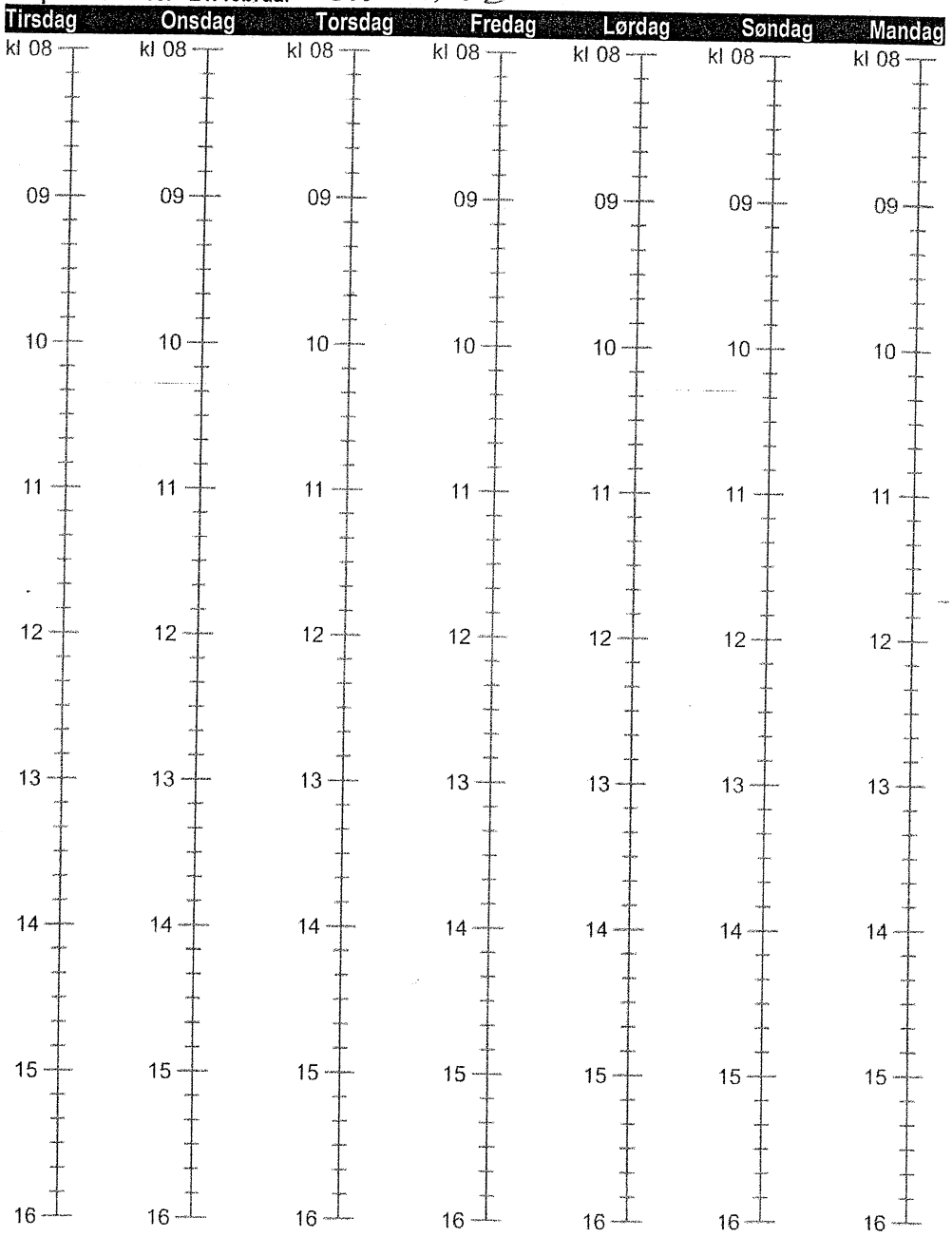
Paper III

Paper IV

Appendix I

For å kunne beregne din individuelle UV-lyseksponering ber vi deg merke av på aksene nedenfor når du har oppholdt deg utendørs. Skjemaet skal levers til Øyvind Aas samme dag som du tar blodprøven.

Tidspunkt : ~~15. - 21. februar~~ 8/2 - 15/2



Blodprøve tatt dato: ..... (Fylles ut av Øyvind Aas)

Vi ber deg om å svare så nøye som mulig på dette spørreskjemaet samme dag som du tar blodprøven. Skjemaet skal levers til Øyvind Aas.

Hvor mye har du vært ute i dagslys i løpet av den siste uken? ..... antall timer

Har du vært i solarium i løpet av de siste to mnd.?

☐ nei ☐ 1-2 ganger ☐ 3+ ganger

Har du vært på solferie i utlandet i løpet av de siste to mnd.?

☐ nei
☐ ja,
Hvis ja, hvor lenge? Antall dager.....

Dato: .....(fylles ut av Øyvind Aas)

Blodprøveinnsamlingen går mot slutten og vi takker for innsatsen! Fiskelever inneholder store mengder D-vitamin. Skrei-sesongen går også mot slutten og vi ber deg svare på spørsmålene nedenfor. Når du har gjort det kan du levere det til Øyvind Aas.

FISKELEVER

Hvor ofte har du spist torskelever denne vinteren?

☐ aldri
☐ 1 gang per
☐ 2-3 ganger
☐ 2-3 ganger per mnd
☐ 1 gang per uke
☐ 2-3 ganger per uke
☐ oftere enn 3 ganger per uke

Dersom du spiser fiskelever, hvor mange spiseskjeer pleier du spise hver gang?

☐ spiser ikke fiskelever
☐ 1 ☐ 2 ☐ 3-4 ☐ 5-6 ☐ 7-10 ☐ 10-13 ☐ 13+

LEVERFETT

Dersom du spiser fiskelever bruker du da også kraften/fettet som leveren er kokt i?

☐ ja ☐ nei

Hvis ja, hvor mye bruker du hver gang?

☐ 1 spise skje
☐ 2 spise skjeer
☐ 0,5 dl
☐ 1 dl
☐ 1,5 dl
☐ + 1,5 dl

Vi ber deg fylle ut dette kostholdsspørreskjemaet så nøye som mulig. Vennligst ta det med og lever det til Øyvind Aas neste gang du skal ta blodprøve.

Alder ..... **Høyde .....** **Vekt .....**

Kjønn ☐ kvinne

☐ mann

FISKELEVER

I sesongen for fiskelever hvor ofte spiser du torskelever ?

☐ aldri
☐ 1 gang per sesong
☐ 2-3 ganger per sesong
☐ 2-3 ganger per mnd
☐ 1 gang per uke
☐ 2-3 ganger per uke
☐ oftere enn 3 ganger per uke

I sesongen for fiskelever hvor ofte spiser du seilever ?

☐ aldri
☐ 1 gang per sesong
☐ 2-3 ganger per sesong
☐ 2-3 ganger per mnd
☐ 1 gang per uke
☐ 2-3 ganger per uke
☐ oftere enn 3 ganger per uke

Dersom du spiser fiskelever, hvor mange spiseskjeer pleier du spise hver gang?

☐ spiser ikke fiskelever
☐ 1 ☐ 2 ☐ 3-4 ☐ 5-6 ☐ 7-10 ☐ 10-13 ☐ 13+

Dersom du spiser fiskelever bruker du da også kraften/fettet som leveren er kokt i?

☐ ja ☐ nei

Hvis ja, hvor mye bruker du hver gang?

☐ 1 spise skje
☐ 2 spise skjeer
☐ 0,5 dl
☐ 1 dl
☐ 1,5 dl
☐ + 1,5 dl

Appendix II

Vi ber deg om å svare så nøye som mulig på dette spørreskjemaet, helst samme dag som du tar blodprøven. Skjemaet skal levers på Borealis.

Hvor mye har du vært ute i dagslys i løpet av den siste uken? ..... antall timer

Har du brukt solkrem når du har vært ute i dagslys?

☐ nei
☐ ja,
Hvis ja, hvor ofte?
☐ alltid ☐ av og til ☐ sjelden

Har du vært i solarium i løpet av de siste to mnd.?

☐ nei ☐ 1-2 ganger ☐ 3+ ganger

Har du vært på solferie i utlandet i løpet av de siste to mnd.?

☐ nei
☐ ja,
Hvis ja, hvor lenge? Antall dager.....

Dato: .....(fylles ut av Janne på Borealis)

Vi ber deg fylle ut dette kostholdsspørreskjemaet så nøye som mulig. Vennligst ta det med og lever det til Janne på Borealis bedriftshelse når du skal avgi blodprøven.

Alder ..... **Høyde .....** **Vekt .....**

Kjønn

☐ kvinne ☐ mann

I sesongen for fiskelever hvor ofte spiser du torskelever ?
☐ aldri
☐ 1 gang per sesong
☐ 2-3 ganger per sesong
☐ 2-3 ganger per mnd
☐ 1 gang per uke
☐ 2-3 ganger per uke
☐ oftere enn 3 ganger per uke

I sesongen for fiskelever hvor ofte spiser du seilever ?
☐ aldri
☐ 1 gang per sesong
☐ 2-3 ganger per sesong
☐ 2-3 ganger per mnd
☐ 1 gang per uke
☐ 2-3 ganger per uke
☐ oftere enn 3 ganger per uke

Dersom du spiser fiskelever, hvor mange spiseskjeer pleier du spise hver gang?
☐ spiser ikke fiskelever
☐ 1 ☐ 2 ☐ 3-4 ☐ 5-6 ☐ 7-10 ☐ 10-13 ☐ 13+

Dersom du spiserer fiskelever bruker du da også kraften/skyen som leveren er kokt i?
☐ ja ☐ nei

Hvis ja, hvor mye bruker du hver gang?
☐ 1 spise skje
☐ 2 spise skjeer
☐ 0,5 dl
☐ 1 dl
☐ 1,5 dl
☐ + 1,5 d

Hvor mange "mås-egg" pleier du å spise per sesong?

.... stykk

Hvor ofte spiser du kveite?
☐ aldri/sjelden
☐ 1-2 gang i løpet av 3 måneder
☐ 1 gang per mnd
☐ 2-3 ganger per mnd
☐ 1 ganger per uke
☐ 2 ganger eller oftere per uke

Appendix III

Kosthold

Vi er interessert i å få kjennskap til hvordan kostholdet ditt er vanligvis. Kryss av for hvert spørsmål om hvor ofte du i gjennomsnitt siste året har brukt den aktuelle matvaren, og hvor mye du pleier å spise/drikke hver gang.

Hvor mange glass melk drikker du vanligvis av hver type? (Sett ett kryss pr. linje)

	aldri/ sjelden	1-4 pr. uke	5-6 pr. uke	1 pr. dag	2-3 pr. dag	4+ pr. dag
Helmelk (søt, sur)	☐	☐	☐	☐	☐	☐
Lettmelk (søt, sur)	☐	☐	☐	☐	☐	☐
Ekstra lettmelk	☐	☐	☐	☐	☐	☐
Skummet (søt, sur)	☐	☐	☐	☐	☐	☐

Hvor mange kopper kaffe drikker du vanligvis av hver sort? (Sett ett kryss for hver linje)

	aldri/ sjelden	1-6 pr. uke	1 pr. dag	2-3 pr. dag	4-5 pr. dag	6-7 pr. dag	8+ pr. dag
Kokekaffe	☐	☐	☐	☐	☐	☐	☐
Traktekaffe	☐	☐	☐	☐	☐	☐	☐
Pulverkaffe	☐	☐	☐	☐	☐	☐	☐

Hvor mange glass juice, saft og brus drikker du vanligvis? (Sett ett kryss for hver linje)

	aldri/ sjelden	1-3 pr. uke	4-6 pr. uke	1 pr. dag	2-3 pr. dag	4+ pr. dag
Appelsinjuice	☐	☐	☐	☐	☐	☐
Saft/brus med sukker	☐	☐	☐	☐	☐	☐
Saft/brus sukkerfri	☐	☐	☐	☐	☐	☐

Hvor ofte spiser du yoghurt (1 beger)? (Sett ett kryss)

☐ aldri/sjelden ☐ 1 pr. uke ☐ 2-3 pr. uke ☐ 4+ pr. uke

Hvor ofte har du i gjennomsnitt siste året spist kornblanding, havregryn eller müsli? (Sett ett kryss)

☐ aldri/nesten aldri ☐ 1-3 pr. uke ☐ 4-6 pr. uke ☐ 1 pr. dag

Hvor mange skiver brød/rundstykker og knekkebrød/skonrokker spiser du vanligvis?
(1/2 rundstykke = 1 brødskive) (Sett ett kryss for hver linje)

	aldri/ sjelden	1-4 pr. uke	5-7 pr. uke	2-3 pr. dag	4-5 pr. dag	6+ pr. dag
Grovt brød						
Fint brød						
Knekkebrød o.l.						

Nedenfor er det spørsmål om bruk av ulike påleggstyper. Vi spør om hvor mange brødskiver med det aktuelle pålegget du pleier å spise. Dersom du også bruker matvarene i andre sammenhenger enn til brød (f. eks. til vafler, frokostblandinger, grøt), ber vi om at du tar med dette når du besvarer spørsmålene.

På hvor mange brødskiver bruker du? (Sett ett kryss pr. linje)

	0 pr. uke	1-3 pr. uke	4-6 pr. uke	1 pr. dag	2-3 pr. dag	4+ pr. dag
Syltetøy og annet søtt pålegg						
Brun ost, helfet						
Brun ost, halvfet/mager						
Hvit ost, helfet						
Hvit ost, halvfet/mager						
Kjøttpålegg, leverpostei						

Videre kommer spørsmål om fiskepålegg.
På hvor mange brødskiver pr. uke har du i gjennomsnitt siste året spist? (Sett ett kryss pr. linje)

	0 pr. uke	1 pr. uke	2-3 pr. uke	4-6 pr. uke	7-9 pr. uke	10+ pr. uke
Makrell i tomat, røkt makrell						
Kaviar						
Annet fiskepålegg						

Hva slags fett bruker du vanligvis på brødet?
(Sett gjerne flere kryss)

☐ bruker ikke fett på brødet
☐ smør
☐ hard margarin (f. eks. Per, Melange)
☐ myk margarin (f. eks. Soft)
☐ smørblandet margarin (f. eks. Bremykt)
☐ Brelett
☐ lettmargarin (f. eks. Soft light, Letta)

Dersom du bruker fett på brødet, hvor tykt lag pleier du smøre på? (En kuvertpakke med margarin veier 12 gram).
(Sett ett kryss)

☐ skrapet (3 g) ☐ tynt lag (5 g) ☐ godt dekket (8 g)
☐ tykt lag (12 g)

Hvor ofte spiser du frukt? (Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr. mnd	1 pr. uke	2-4 pr. uke	5-6 pr. uke	1 pr. dag	2+ pr. dag
Epler/pærer							
Appelsiner o.l.							
Bananer							
Annen frukt (f.eks. druer, fersken)							

Hvor ofte spiser du ulike typer grønnsaker?
(Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr. mnd	1 pr. uke	2 pr. uke	3 pr. uke	4-5 pr. uke	6-7 pr. uke
Gulrøtter							
Kål							
Kålrot							
Broccoli/blomkål							
Blandet salat							
Grønnsakblanding (frossen)							
Andre grønnsaker							

For de grønnsakene du spiser, kryss av for hvor mye du spiser hver gang. (Sett ett kryss for hver sort)

- gulrøtter ☐ 1/2 stk. ☐ 1 stk. ☐ 1 1/2 stk. ☐ 2+ stk.
- kål ☐ 1/2 dl ☐ 1 dl ☐ 1 1/2 dl ☐ 2+ dl
- kålrot ☐ 1/2 dl ☐ 1 dl ☐ 1 1/2 dl ☐ 2+ dl
- broccoli/blomkål ☐ 1-2 buketter ☐ 3-4 buketter ☐ 5+ buketter
- blandet salat ☐ 1 dl ☐ 2 dl ☐ 3 dl ☐ 4+ dl
- grønnsakblanding ☐ 1/2 dl ☐ 1 dl ☐ 2 dl ☐ 3+ dl

Hvor mange poteter spiser du vanligvis (kokte, stekte, mos)? (Sett ett kryss)

☐ spiser ikke/spiser sjelden poteter

☐ 1-4 pr. uke ☐ 5-6 pr. uke

☐ 1 pr. dag ☐ 2 pr. dag

☐ 3 pr. dag ☐ 4+ pr dag

Hvor ofte bruker du ris og spaghetti/makaroni ?
(Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr. mnd	1 pr. uke	2 pr. uke	3+ pr. uke
Ris					
Spaghetti, makaroni					

Hvor ofte spiser du risengrynsgrøt? (Sett ett kryss)

☐ aldri/sjelden ☐ 1 pr. mnd ☐ 2-3 pr. mnd ☐ 1+ pr. uke

Hva slags fett blir vanligvis brukt til matlaging i din husholdning? (Sett gjerne flere kryss)

☐ smør

☐ hard margarin (f. eks. Per, Melange)

☐ myk margarin (f. eks. Soft)

☐ smørblandet margarin (f. eks. Bremykt)

☐ soyaolje ☐ olivenolje ☐ maisolje

Fisk

Vi vil gjerne vite hvor ofte du pleier å spise fisk, og ber deg fylle ut spørsmålene om fiskeforbruk så godt du kan. Tilgangen på fisk kan variere gjennom året. Vær vennlig å markere i hvilke årstider du spiser de ulike fiskeslagene.

	aldri/ sjelden	like mye hele året	vinter	vår	sommer	høst
Torsk, sei, hyse, lyr						
Steinbit, flyndre, uer						
Laks, ørret						
Makrell						
Sild						

Med tanke på de periodene av året der du spiser fisk, hvor ofte pleier du å spise følgende? (Sett ett kryss pr. linje)

	aldri/ sjelden	1 pr. mnd	2-3 pr. mnd	1 pr. uke	2 pr. uke	3+ pr. uke
Kokt torsk, sei, hyse, lyr						
Stekt torsk, sei, hyse, lyr						
Steinbit, flyndre, uer						
Laks, ørret						
Makrell						
Sild						

Dersom du spiser fisk, hvor mye spiser du vanligvis pr. gang? (1 skive/stykke = 150 gram)
(Sett ett kryss for hver linje)

Kokt fisk (skive) ☐ 1 ☐ 1,5 ☐ 2 ☐ 3 ☐ 4+

Stekt fisk (stykke) ☐ 1 ☐ 1,5 ☐ 2 ☐ 3 ☐ 4+

Hvor ofte bruker du følgende typer fiskemat?
(Sett ett kryss pr. linje)

	aldri/ sjelden	1 pr. mnd	2-3 pr. mnd	1 pr. uke	2+ pr. uke
Fiskekaker/pudding/ boller					
Plukkfisk, fiskegrateng					
Frityrfisk, fiskepinner					
Andre fiskeretter					

Hvor stor mengde pleier du vanligvis å spise av de ulike rettene? (Sett ett kryss for hver linje)

- fiskekaker/pudding/boller (stk.) (2 fiskeboller=1 fiskekake) ☐ 1 ☐ 2 ☐ 3 ☐ 4+
- plukkfisk, fiskegrateng (dl) ☐ 1-2 ☐ 3-4 ☐ 5+
- frityrfisk, fiskepinner (stk.) ☐ 1-2 ☐ 3-4 ☐ 5-6 ☐ 7+

Hvor ofte spiser du skalldyr (f. eks. reker, krabbe)? (Sett ett kryss)

aldri/ sjelden	1 pr. mnd	2-3 pr mnd	1+ pr. uke
☐	☐	☐	☐

I tillegg til informasjon om fiskeforbruk er det viktig å få kartlagt hvilket tilbehør som blir servert til fisk. Hvor ofte bruker du følgende til fisk? (Sett ett kryss pr. linje)

	aldri/ sjelden	1 pr. mnd	2-3 pr. mnd	1 pr. uke	2+ pr. uke
Smeltet eller fast margarin/fett					
Seterrømme (35%)					
Lettrømme (20%)					
Saus med fett (hvit/brun)					
Saus uten fett (hvit/brun)					

For de ulike typene tilbehør du bruker til fisk, vær vennlig å kryss av for hvor mye du vanligvis pleier spise.

- smeltet/fast fett (ss) ☐ 1/2 ☐ 1 ☐ 2 ☐ 3 ☐ 4+
- seterrømme (ss) ☐ 1/2 ☐ 1 ☐ 2 ☐ 3 ☐ 4+
- lettrømme (ss) ☐ 1/2 ☐ 1 ☐ 2 ☐ 3 ☐ 4+
- saus med fett (dl) ☐ 1/4 ☐ 1/2 ☐ 3/4 ☐ 1 ☐ 2+
- saus uten fett (dl) ☐ 1/4 ☐ 1/2 ☐ 3/4 ☐ 1 ☐ 2+

Andre matvarer

Hvor ofte spiser du følgende kjøtt- og fjærkreretter? (Sett ett kryss for hver rett)

	aldri/ sjelden	1 pr. mnd	2-3 pr. mnd	1 pr. uke	2+ pr. uke
Steik (okse, svin, får)					
Koteletter					
Biff					
Kjøttkaker, karbonader					
Pølser					
Gryterett, lapskaus					
Pizza m/kjøtt					
Kylling					
Andre kjøttretter					

Dersom du spiser følgende retter, oppgi mengden du vanligvis spiser: (Sett ett kryss for hver linje)

- steik (skiver) ☐ 1 ☐ 2 ☐ 3 ☐ 4+
- koteletter (stk.) ☐ 1/2 ☐ 1 ☐ 1,5 ☐ 2+
- kjøttkaker, karbonader (stk.) ☐ 1 ☐ 2 ☐ 3 ☐ 4+
- pølser (stk. à 150g) ☐ 1/2 ☐ 1 ☐ 1,5 ☐ 2+
- gryterett, lapskaus (dl) ☐ 1-2 ☐ 3 ☐ 4 ☐ 5+
- pizza m/kjøtt (stykke à 100 g) ☐ 1 ☐ 2 ☐ 3 ☐ 4+

Hvor mange egg spiser du vanligvis i løpet av en uke (stekte, kokte, eggerøre, omelett)? (Sett ett kryss)

☐ 0 ☐ 1 ☐ 2 ☐ 3-4 ☐ 5-6 ☐ 7+

Vi ber deg fylle ut hovedrettene til middag en gang til som en oppsummering. Kryss av i den ruten som passer hvor ofte du i gjennomsnitt i løpet av siste år har spist slik mat til middag

	5+ pr. uke	4 pr. uke	3 pr. uke	2 pr. uke	1 pr. uke	2-3 pr. mnd	1 pr. mnd	nesten aldri
Rent kjøtt	☐	☐	☐	☐	☐	☐	☐	☐
Oppmalt kjøtt	☐	☐	☐	☐	☐	☐	☐	☐
Fet fisk (makrell, laks o.l.)	☐	☐	☐	☐	☐	☐	☐	☐
Mager fisk (torsk o.l.)	☐	☐	☐	☐	☐	☐	☐	☐
Fiskemat	☐	☐	☐	☐	☐	☐	☐	☐

Hvor ofte spiser du iskrem (til dessert, krone-is osv.)? (Sett ett kryss for hvor ofte du spiser iskrem om sommeren, og ett kryss for resten av året)

	aldri/ sjelden	1-3 pr mnd	1 pr. uke	2-3 pr. uke	4+ pr. uke
– om sommeren	☐	☐	☐	☐	☐
– resten av året	☐	☐	☐	☐	☐

Hvor mye is spiser du vanligvis pr. gang? (Sett ett kryss)

☐ 1 dl ☐ 2 dl ☐ 3 dl ☐ 4+ dl

Hvor ofte spiser du bakervarer som boller, kaker, wienerbrød, vafler, småkaker? (Sett ett kryss)

	aldri/ sjelden	1-3 pr. mnd	1 pr. uke	2-3 pr. uke	4-6 pr. uke	7+ pr. uke
Gjærbakst(boller)						
Kaker						
Pannekaker						
Vafler						
Småkaker						

Hvor ofte spiser du dessert? (Sett ett kryss)

	aldri/ sjelden	1-3 pr. mnd	1 pr. uke	2-3 pr uke	4-6 pr. uke	7+ pr. uke
Pudding Sjokolade/karamell						
Riskrem, fromasj						
Kompott, fruktgrøt hermetisk frukt						

Hvor ofte spiser du sjokolade? (Sett ett kryss)

☐ aldri/sjelden ☐ 1-3 pr. mnd ☐ 1 pr. uke
☐ 2-3 pr. uke ☐ 4-6 pr. uke ☐ 1+ pr. dag

Dersom du spiser sjokolade, hvor mye pleier du vanligvis å spise hver gang? Tenk deg størrelsen på en Kvikk-Lunsj sjokolade, og oppgi hvor mye du spiser i forhold til den.

☐ 1/4 ☐ 1/2 ☐ 3/4 ☐ 1 ☐ 1,5 ☐ 2+

Hvor ofte spiser du salt snacks? (Sett ett kryss)

	aldri/ sjelden	1-3 pr. mnd	1 pr. uke	2-3 pr. uke	4-6 pr. uke	7+ pr. uke
Potetchips						
Peanøtter						

Tilberedningsmåte

Har du mikrobølgeovn? ☐ Ja ☐ Nei

Hvis Ja; hvor mange ganger pr. uke bruker du mikrobølgeovnen til

	ganger pr. uke
middagslaging?	.....
annet?	.....

Hvilken farve foretrekker du på stekeskorpen?

☐ Lys brun ☐ Middels ☐ Mørk brun

Hvor ofte spiser du stekt eller grillet mat?

	aldri/ sjelden	1-3 pr. mnd	1 pr. uke	2-3 pr. uke	4-6 pr. uke	7+ pr. uke
Mørkt kjøtt (biff ol.)						
Lyst kjøtt (kylling ol.)						
Oppmalt kjøtt (kjøttkaker ol.)						
Bacon						
Fisk						

Bruker du stekefettet eller sjyen etter steking?

☐ nei, aldri ☐ av og til
☐ som oftest ☐ ja, alltid

Tran og fiskeoljekapsler

Bruker du tran (flytende)? ☐ Ja ☐ Nei

Hvis ja; hvor ofte tar du tran?
Sett ett kryss for hver linje.

	aldri/ sjelden	1-3 pr. mnd	1 pr. uke	2-6 pr. uke	daglig
- om vinteren	☐	☐	☐	☐	☐
- resten av året	☐	☐	☐	☐	☐

Hvor mye tran pleier du å ta hver gang?

☐ 1 ts ☐ 1/2ss ☐ 1+ss

Bruker du tranpiller/kapsler? ☐ Ja ☐ Nei

Hvis ja; hvor ofte tar du tranpiller/kapsler?
Sett ett kryss for hver linje.

	aldri/ sjelden	1-3 pr. mnd	1 pr. uke	2-6 pr. uke	daglig
- om vinteren	☐	☐	☐	☐	☐
- resten av året	☐	☐	☐	☐	☐

Hvilken type tranpiller/kapsler bruker du vanligvis, og hvor mange pleier du å ta hver gang?

	ja	antall pr. gang
Møllers Basic	☐	.....
Møllers dobbel	☐	.....
annet, navn	☐	.....

Bruker du fiskeoljekapsler? ☐ Ja ☐ Nei

Hvis ja; hvor ofte tar du fiskeoljekapsler?

aldri/ sjelden	1-3 pr. mnd	1 pr. uke	2-6 pr. uke	daglig
☐	☐	☐	☐	☐

Kosttilskudd

Bruker du annet kosttilskudd (eks. vitaminer, mineraler)? ☐ Ja ☐ Nei

Hvis ja; hvor ofte tar du slike kosttilskudd?

aldri/ sjelden	1-3 pr. mnd	1 pr. uke	2-6 pr. uke	daglig
☐	☐	☐	☐	☐

Navn .....

Alkohol

Er du total avholdskvinne/mann? ☐ Ja ☐ Nei

Hvis Nei, hvor ofte og hvor mye drakk du i gjennomsnitt siste året? (Sett ett kryss for hver linje)

	aldri/ sjelden	1 pr. mnd	2-3 pr. mnd	1 pr. uke	2-4 pr. uke	5-6 pr. uke	1+ pr. dag
Øl (½ L)	☐	☐	☐	☐	☐	☐	☐
Vin (glass)	☐	☐	☐	☐	☐	☐	☐
Brennevin (drinker)	☐	☐	☐	☐	☐	☐	☐

Solvaner

Dersom du i begynnelsen av sommeren soler deg kraftig, blir huden din; (sett ett kryss)

☐ brun uten først å være rød ☐ rød

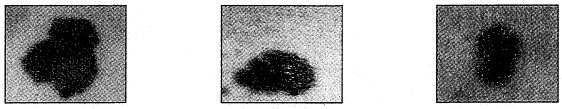
☐ rød med svie ☐ rød med svie og blemmer

Etter gjentatt og lenge soling, blir huden din; (sett ett kryss)

☐ dypt brun ☐ brun ☐ lys brun ☐ aldri brun

Hvor mange uregelmessige føflekker større enn 5 mm har du sammenlagt på begge beina (fra tærne til lysken)? Tre eksempler på føflekker større enn 5 mm med uregelmessig form er vist i nedenfor.

☐ 0 ☐ 1 ☐ 2-3 ☐ 4-6 ☐ 7-12 ☐ 13-24 ☐ 25+



Hvor mange små, regelmessige føflekker har du sammenlagt på begge beina (fra tærne til lysken)?

☐ 0 ☐ 1-10 ☐ 11-50 ☐ 51+

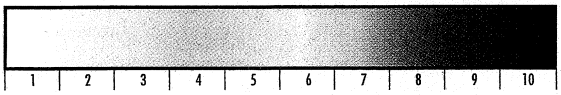
Hvilken øyefarge har du? (sett ett kryss)

☐ brun ☐ grå, grønn eller blanding ☐ blå

Hva er din opprinnelige hårfarge? (sett ett kryss)

☐ mørkbrunt, svart ☐ brun ☐ blond, gul ☐ rød

For å kunne studere effekten av soling på risiko for hudkreft ber vi deg gi opplysninger om hudfarge
Sett ett kryss på den fargen som best passer din hudfarge (uten soling)



Hvor ofte dusjer eller bader du?

	Mer enn 1 g dagl	1 g dagl	4-6 g pr. uke	2-3 g pr. uke	1 g pr. uke	2-3 g pr. mnd.	Sjelden aldri
Med såpe/shampo							
Uten såpe/shampo							

Hvor mange ganger pr. år er du blitt forbrent av solen slik at du har fått svie og blemmer med avflassing etterpå? (ett kryss for hver aldersgruppe)

Alder	Aldri	Høyst 1 gang pr. år	2-3 g. pr. år	4-5 g. pr. år	6 eller flere ganger
Før 10 år					
10-19 år					
20-44 år					
45+ år					

Hvor mange uker soler du deg pr. år i syden?

Alder	Aldri	1 uke	2-3 uker	4-5 uker	7 uker eller mer
Før 10 år					
10-19 år					
20-45 år					
45+ år					

Hvor mange uker pr. år soler du deg i Norge eller utenfor syden?

Alder	Aldri	1 uke	2-3 uker	4-5 uker	7 uker eller mer
Før 10 år					
10-19 år					
20-45 år					
45+ år					

Når bruker du krem med solfaktor (sett evt. flere kryss):

☐ påsken ☐ i Norge eller utenfor syden ☐ solferie i syden

Hvilke solfaktorer bruker du i disse periodene?

	påsken	i Norge eller utenfor syden	solferie i syden
- I dag	.....	.....	.....
- For 10 år siden	.....	.....	.....

Hvilke solkremmerker bruker du? Angi faktor hvis du husker.

	Ja	faktor
Piz Buin	☐	.....
Ambre Solairé	☐	.....
Delial	☐	.....
Nivea	☐	.....
Natusan	☐	.....
HTH	☐	.....
Cosmica	☐	.....
Andre.....	☐	.....

Hvor ofte har du solt deg i solarium?

Alder	Aldri	Sjelden	1 gang pr. mnd.	2 ganger pr. mnd.	3-4 ganger pr. mnd	oftere enn1 gang pr. uke
Før 10 år						
10-19 år						
20-44 år						
45+ år						

Til slutt vil vi spørre deg om ditt samtykke til å kontakte deg på nytt pr. post. Vi vil hente adressen fra det sentrale personregister. ☐ Ja ☐ Nei

Takk for at du ville delta i undersøkelsen

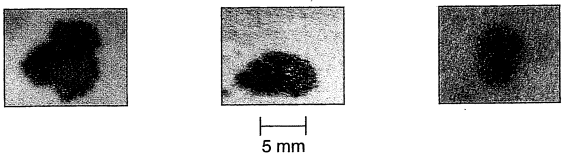
Solvaner

Får du fregner når du soler deg? ☐ Ja ☐ Nei

Hvor mange føflekker har du sammenlagt på begge armer (fra fingertuppene til skuldrene)?

☐ 0 ☐ 1-10 ☐ 11-50 ☐ 51+

Hvor mange uregelmessige føflekker større enn 5 mm har du sammenlagt på begge armene (fra fingrene til armhulene)? Tre eksempler på føflekker større enn 5 mm med uregelmessig form er vist i nedenfor.



☐ 0 ☐ 1 ☐ 2-3 ☐ 4-6 ☐ 7-12 ☐ 13-24 ☐ 25+

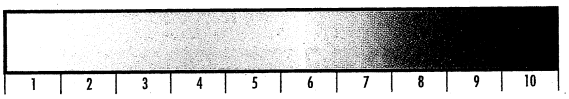
Hvor mange små, regelmessige føflekker har du sammenlagt på begge armene (fra fingrene til armhulene)?

☐ 0 ☐ 1-10 ☐ 11-50 ☐ 51+

Hva er din opprinnelige hårfarge? (sett ett kryss)

☐ mørkbrunt, svart ☐ brun ☐ blond, gul ☐ rød

For å kunne studere effekten av soling på risiko for hudkreft ber vi deg gi opplysninger om hudfarge
Sett ett kryss på den fargen som best passer din hudfarge (uten soling)



Hvor ofte dusjer eller bader du?

	Mer enn 1 g dagl	1 g dagl	4-6 g pr. uke	2-3 g pr. uke	1 g pr. uke	2-3 g pr. mnd.	Sjelden aldri
Med såpe/shampo							
Uten såpe/shampo							

Hvor mange ganger pr. år er du blitt forbrent av solen slik at du har fått svie og blemmer med avflassing etterpå? (ett kryss for hver aldersgruppe)

Årstall	Aldri	Høyst 1 gang pr. år	2-3 g. pr. år	4-5 g. pr. år	6 eller flere ganger
1991-94					
1995-98					

Hvor mange uker soler du deg pr. år i syden?

Årstall	Aldri	1 uke	2-3 uker	4-5 uker	7 uker eller mer
1991-94					
1995-98					

Hvor mange uker pr. år soler du deg i Norge eller utenfor syden?

Årstall	Aldri	1 uke	2-3 uker	4-5 uker	7 uker eller mer
1991-94					
1995-98					

Når bruker du krem med solfaktor (sett evt. flere kryss):

☐ påsken ☐ i Norge eller utenfor syden ☐ solferie i syden

Hvilke solfaktorer bruker du i disse periodene?

	påsken	i Norge eller utenfor syden	solferie i syden
- I dag	.....	.....	.....
- For 10 år siden	.....	.....	.....

Hvilke solkremmerker bruker du? Angi faktor hvis du husker.

	Ja	faktor		Ja	faktor
Piz Buin	☐	....	Cosmica	☐	....
Ambre Solairé	☐	....	Natusan	☐	....
HTH	☐	....	Delial	☐	....
Andre, angi navn.....		....			

Hvor ofte har du solt deg i solarium?

Alder	Aldri	Sjelden	1 gang pr. mnd.	2 ganger pr. mnd.	3-4 ganger pr. mnd	oftere enn1 gang pr. uke
1991-94						
1995-98						

Til slutt vil vi spørre deg om ditt samtykke til å kontakte deg på nytt pr. post.
Vi vil hente adressen fra det sentrale personregister.

☐ Ja ☐ Nei

Takk for at du ville delta i undersøkelsen

Kosthold

For hver matsort nedenfor ber vi deg krysse av i den ruten som passer hvor ofte du i gjennomsnitt i løpet av siste år har spist slik mat.

	6-10 pr dag	4-5 pr dag	2-3 pr dag	1 pr dag	5-6 pr uke	2-4 pr uke	1 pr uke	1-3 pr måned	Nesten aldri
Helmelk (glass)									
Skummet melk (glass)									
Lettmelk (glass)									
Kokekaffe (kopper)									
Traktekaffe (kopper)									
Pulverkaffe (kopper)									
Grov brød (skiver)									
Fint brød (skiver)									
Ost (skiver)									
Poteter									
Epler/pærer									
Appelsiner o.l.									

Middag	6-7 pr uke	4-5 pr uke	3 pr uke	2 pr uke	1 pr uke	2-3 pr måned	1 pr måned	Nesten aldri
Rent kjøtt								
Oppmalt kjøtt								
Fet fisk (makrell,laks o.l)								
Mager fisk (torsk ol.)								
Ris, spaghetti								
Gulerøtter								
Kål								
Kålrot								
Salat								
Broccoli/Blomkål								

Hva slags fett blir vanligvis brukt i din husholdning?

	På brød	Til matlaging
Smør eller hard margarin		
Myk (soft) margarin eller olje		
Smør/margarin blanding		

Hvor mye melk drakk du som barn hver dag?

☐ drakk ikke melk ☐ 1-3 glass ☐ 4-6 glass ☐ 7 glass eller mer

Hvor ofte spiste du grønnsaker til middag som barn?

☐ aldri ☐ 1 gang i uken eller mer sjelden

☐ 2-3 ganger i uken ☐ 4 eller flere ganger

Alkohol

Er du total avholdskvinne? Ja ☐ Nei ☐

Hvis Nei, hvor ofte og hvor mye drakk du i gjennomsnitt siste året?

	6-10 pr dag	4-5 pr dag	2-3 pr dag	1 pr dag	5-6 pr uke	2-4 pr uke	1 pr uke	1-3 pr måned	Nesten aldri
Øl (1/2 liter)									
Vin (glass)									
Brennevin (drinker)									

Solvaner

Dersom du i begynnelsen av sommeren soler deg kraftig, blir huden din; (Sett ett kryss)

☐ brun uten å først være rød ☐ rød

☐ rød med svie ☐ rød med svie og blemmer

Etter gjentatt og lenge soling, blir huden din; (Sett ett kryss)

☐ dypt brun ☐ brun ☐ lys brun ☐ aldri brun

Hvor mange uregelmessige føflekker større enn 5 mm har du sammenlagt på begge beina (fra tærne til lysken)?

(På siste side av brosjyren er det bilder som viser hva vi mener med uregelmessige føflekker.)

☐ 0 ☐ 1 ☐ 2-3 ☐ 4-6 ☐ 7-12 ☐ 13-24 ☐ 25+

Hvilken øyefarve har du? (Sett ett kryss)

☐ brun ☐ grå, grønn eller blanding ☐ blå

Hvilken hårfarve har du? (Sett ett kryss)

☐ mørkbrun, svart ☐ brun ☐ blond, gul ☐ rød

Hvor mange ganger pr. år er du blitt forbrent av solen slik at du har fått svie eller blemmer med avflassing etterpå? (Ett kryss for hver aldersgruppe)

Alder	Aldri	Høyst 1 gang pr.år	2-3 g. pr. år	4-5 g. pr. år	6 eller flere ganger
Før 10 år					
10-19 år					
20-29 år					
30-39 år					
40-49 år					

Hvor mange uker i gjennomsnitt pr. år har du vært på badeferie i syden eller i Norge?

Alder	Aldri	1 uke	2-3 uker	4-6 uker	7 uker eller mer
Før 10 år					
10-19 år					
20-29 år					
30-39 år					
40-49 år					

Hvor ofte har du solt deg i solarium?

Alder	Aldri	Sjelden	1 gang pr. mnd.	2 gang pr. mnd.	3-4 gang pr. mnd.	oftere enn 1 gang pr. uke
Før 10 år						
10-19 år						
20-29 år						
30-39 år						
40-49 år						

Takk for at du ville delta i undersøkelsen!

ISBN 978-82-9263-27-5