Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences
Department of Public Health and Nursing

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Serum 25-hydroxyvitamin D level in relation to weight change and risk of weight gain in a prospective cohort of Norwegian adults:

The HUNT Study

Master's thesis in Public Health, specializing in Global Health
Supervisor: Yi-Qian Sun & Xiao-Mei Mai
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ABSTRACT

Introduction: Overweight and obesity have long been associated with a variety of adverse health outcomes. In recent years, these health concerns have risen to epidemic proportions worldwide. Meanwhile, low vitamin D status remains a potential threat to health globally. Although cross-sectional studies have indicated an association between low vitamin D status and obesity, the longitudinal association between vitamin D status and weight change remains unclear. We sought to investigate the relationship of serum 25-hydroxyvitamin D [25(OH)D] levels with weight change and risk of weight gain in an adult population after 11 years of follow-up.

Methods: This prospective study included 1,501 adults who participated in the second and third surveys of the Nord-Trøndelag Health Study [HUNT2 (1995-1997) and HUNT3 (2006-2008)] in Norway. They were ≥19 years of age and had a normal body mass index (BMI ≥18.5 and <25 kg/m^2) at baseline. Serum 25(OH)D levels were determined at baseline and classified as <25, 25.0-49.9, 50.0-74.9, and ≥75 nmol/L. Comprehensive lifestyle and anthropometric data were collected at baseline and follow-up. Percentage annual weight change was calculated using participant weight change and follow-up time between HUNT2 and HUNT3. Annual weight gain was defined as percentage annual weight change >1.25%, while clinical weight gain was defined as weight change ≥5% over follow-up. Adjusted coefficients and 95% confidence intervals (CI) for the association of baseline 25(OH)D level with percentage annual weight change were estimated using multivariable linear regression. Multivariable logistic regression models were used to estimate adjusted odds ratios (OR) and 95% CIs for risk of annual weight gain and risk of clinical weight gain. We evaluated effect modification by sex, age, and physical activity for risk of clinical weight gain.

Results: Over the follow-up period, a reduction in percentage annual weight change seemed to be present but not statistically significant as 25(OH)D increased by 25 nmol/L increments (*coefficient* = -0.05, 95% CI: -0.11–0.01). In total, 201 participants experienced annual weight gain (13.4%) and 708 experienced clinical weight (47.2%). Risk of annual weight gain and risk of clinical weight gain were significantly reduced in the 25(OH)D ≥75 nmol/L group compared with the <25 nmol/L group (OR=0.32, 95% CI: 0.12–0.87 and OR=0.46, 95% CI: 0.22–0.97, respectively), and a dose-response relationship was observed between 25(OH)D levels and both outcomes. The association of 25(OH)D with risk of clinical weight gain appeared stronger in female and low physical activity participants compared with male and high physical activity participants (OR=0.70 *vs.* 1.00 and 0.65 *vs.* 0.91, respectively). There was no effect modification by age.

Conclusion: These findings suggested an association of serum 25(OH)D levels with weight change and risk of weight gain. The association appeared to differ by sex and physical activity. Given the prevalence and wide-ranging health effects of both obesity and vitamin D deficiency, the link between vitamin D status and body weight should be considered in public health efforts.

Keywords: obesity; weight change; weight gain; 25-hydroxyvitamin D; vitamin D; HUNT; prospective studies

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ABBREVIATIONS

25(OH)D	25-hydroxyvitamin D
ANOVA	analysis of variance
BMI	body mass index
CI	confidence interval
DAG	directed acyclic graph
HIC	high-income country
HUNT	Nord-Trøndelag Health Study
LMIC	low- and middle-income country
NCD	noncommunicable disease
OR	odds ratio
RCT	randomized controlled trial
REK	Regional Committee for Medical Research Ethics
WHO	World Health Organization

1. INTRODUCTION

1.1 Background

1.1.1 Global burden of overweight & obesity

The World Health Organization (WHO) defines obesity as a preventable disorder characterized by abnormal or excessive fat accumulation that may be detrimental to health [1]. Overweight and obesity are often assessed using body mass index (BMI; kg/m^2). The prevalence of overweight and obesity has increased rapidly in past years. Between 1975 and 2016, the prevalence of obesity has nearly tripled globally, with current estimates identifying 39% of adults as overweight and 13% as obese [1]. In Norway, the prevalence of overweight and obesity is also on the rise [2]. According to a 2017 report by the Norwegian Institute of Public Health, about 25% of young adults are overweight or obese, and the prevalence of obesity is 25 and 21% in middle-aged male and female adults, respectively [2]. High BMI has been linked to increased risk of many noncommunicable diseases (NCDs), such as type 2 diabetes, hypertension, dyslipidemia, coronary heart disease, osteoarthritis, stroke, and respiratory problems [3] as well as some forms of cancers [4]; furthermore, overweight/obesity has been associated with increased risk of all-cause and cardiovascular disease-specific mortality [3]. High BMI accounted for four million deaths worldwide in 2015 alone [2] and is estimated to contribute to 2,400 deaths annually in Norway [5].

While obesity was once considered a public health problem specific to high income countries (HICs), many low- and middle-income countries (LMICs) now face “double burdens” of both over- and undernutrition [6]. Infectious disease remains a large problem in LMICs due in

part to undernutrition; at the same time, the prevalence of NCDs and NCD-related deaths is on the rise [6, 7]. Overweight/obesity and physical inactivity have been identified as major contributors to the NCD burden [7]. In recent years, the concept of "obesogenic" environments has arisen as an explanation of how "surroundings, opportunities, or conditions of life" interact to influence physical activity and food intake, and thus body weight, on population and individual levels [8].

1.1.2 Biological significance of vitamin D

Vitamin D is a fat-soluble vitamin created by the skin after exposure to UVB sunlight, but it can also be obtained from dietary sources like oily fish, fortified milk, and nutritional supplements. It has been estimated that of the vitamin D utilized by the human body, roughly 80% is produced in the skin and 20% is from the dietary sources. However, this ratio can vary based on geographical location, seasonal sun exposure, ethnicity, and nutritional factors [9-11]. Vitamin D_3 (cholecalciferol) is the form of vitamin D produced by the skin and typically derived from animal sources, while vitamin D_2 (ergocalciferol) is found in some fungi [12]. Vitamins D_2 and D_3 are prohormones, meaning that they must undergo a two-step hydroxylation process before becoming biologically active. Vitamin D in either form is first converted to 25-hydroxyvitamin D [25(OH)D] in the liver, followed by conversion of 25(OH)D to the active metabolite 1,25-dihydroxyvitamin D_2 or D_3 (calcitriol) in the kidneys [13].

Vitamin D is essential for skeletal growth, development, and health due to its role in calcium and phosphorus maintenance homeostasis [14, 15]. Vitamin D deficiency has been implicated as the cause of rickets and osteomalacia, metabolic skeletal diseases occurring in children and adults, respectively [12]. Additionally, a growing body of evidence from population

and experimental studies suggests that vitamin D deficiency may be linked to a variety of other medical conditions, including diabetes [16], infection [17], and several cancers [18]. This may be explained by hypothesized roles of vitamin D in immune system regulation and cell cycle progression [13]. Besides its conventional pathways, vitamin D synthesis enzymes and vitamin D receptors are present in many tissues, including in cells of the digestive, respiratory, immune, and reproductive systems [19-21]. Vitamin D is estimated to regulate more than 1% of all gene expression in humans [22], and it can function in an autocrine and paracrine manner [23].

Serum 25(OH)D has been shown to serve as an accurate composite measure of total vitamin D exposure from dietary sources and skin synthesis [13]. There is no definitive consensus on what level of vitamin D is sufficient for human health, but a commonly accepted definition of vitamin D insufficiency has been established by the Institute of Medicine as serum 25(OH)D levels <50 nmol/L [13]. Vitamin D deficiency is generally regarded as serum 25(OH)D levels <25 or <30 nmol/L [13]. Although both types of vitamin D undergo the same metabolic processes, some evidence suggests that vitamin D_3 may be more effective in raising serum 25(OH)D concentrations [24]. In this report, "vitamin D" will be used as a general term in reference to vitamins D_2 and D_3 and relevant metabolites; however, specific references will be made to 25(OH)D as necessary.

1.1.3 Vitamin D in global context

Vitamin D deficiency has been identified as a major global health problem across all life stages [25]. Interestingly, low vitamin D status is present in countries both with access to vitamin-rich foods and with adequate year-round sun exposure [25]. Vitamin D deficiency been identified in 40.4% of the general population in Europe and 26.0% in the United States, with

13.0% and 6.7% reporting serum 25(OH)D concentrations below 30 nmol/L, respectively [26]. In Norway, population studies have found a prevalence of vitamin D deficiency ranging from 15% to 90%, depending on age, location of residence, supplementation, and immigration status [27-30]. At high latitudes, significant seasonal variation is also present; one study reported a 44% difference in prevalence of vitamin D deficiency between summer and winter seasons [30].

Although data from many LMICs are incomplete, the burden of low serum vitamin D level appears to be higher in LMICs than HICs [25, 31-33]. In a recent review of vitamin D status in LMICs, India, Pakistan, Afghanistan, Mongolia, and Tunisia were identified as "hotspots" for vitamin D deficiency [32]. In these countries, the prevalence of 25(OH)D of <20/25/30 nmol/L ranged from 25 to 90%. Similar studies have found the highest prevalence of vitamin D deficiency globally in women and girls in the Middle East [25, 31]. Cultural factors like extensive skin coverage linked to modest dress and decreased synthesis of vitamin D due to high melanin concentration in skin may explain the high proportion of vitamin D deficient individuals in this region [25, 33, 34].

1.1.4 Public health response to vitamin D deficiency

It has been suggested that public health intervention may be warranted if prevalence of vitamin D deficiency exceeds 20% in a total population or vulnerable subpopulation [12]. Subpopulations particularly at risk for vitamin D deficiency have been identified as infants, children, women of child-bearing age, pregnant women, older persons, and non-western immigrants [12, 31]. It is likely that much success in reduction of vitamin D deficiency in HICs can be attributed to consumption of fatty fish, vitamin D supplementation, and fortification of staple foods [25]. Addition of vitamin D to foods such as milk, margarine, and butter began in

the 1930s in Europe and North America, with Norway gradually implementing similar policies between 1950 and 1990 [29]. The effect of vitamin D fortification on serum 25(OH)D level in the Finnish population has been well-described [35, 36]. Following implementation of vitamin D fortification policies, mean serum 25(OH)D levels increased from 48 nmol/L to 65 nmol/L over 11 years of follow-up [36]. It is important to note that vitamin D supplementation also increased from 11% to 41% during the same time period; however, when the analysis was restricted to supplement non-users, a 6 nmol/L higher mean increase in serum 25(OH)D was observed among individuals consuming fortified foods than those who did not [36].

Recently, vitamin D fortification efforts have begun to expand in LMICS on a voluntary basis, including India, Egypt, Afghanistan, Zimbabwe, and some parts of Southeast Asia [32, 37, 38]. As of 2017, however, Morocco was the only LMIC to mandate addition of vitamin D to staple foods, specifically vegetable oils [32]. Vitamin D supplementation in at-risk groups has been proposed as an interim strategy to combat vitamin D deficiency as LMICs work to develop national food fortification programs [12]. In addition to dietary interventions, it has been recommended that public health and medical professionals work to promote healthy exposure to UVB sunlight and increase awareness of the importance of vitamin D in the general population and among policymakers [34].

1.1.5 Possible bidirectional association between overweight/obesity & vitamin D

There may be a bidirectional relationship between vitamin D deficiency and obesity. Obese individuals have lower levels of vitamin D compared with normal weight individuals [39]. Possible explanations for the obesity-vitamin D association include lifestyle differences between obese and non-obese individuals, such as differences in supplement intake and physical activity-

related sun exposure [40]; volumetric dilution of vitamin D serum levels in obese individuals [41]; and sequestration of vitamin D in adipose tissue [42]. It has also been proposed that low vitamin D status may contribute to the development of obesity [43] due to its role in regulation of lipogenesis and lipolysis in the adipose tissue as well as energy metabolism [40]. Additionally, vitamin D enhances insulin sensitivity and reduces appetite [40].

Cross-sectional studies have consistently revealed an association between low vitamin D status and obesity [44-49]. However, longitudinal studies on the vitamin D-obesity association have yielded inconsistent results [27, 50-53]. One prospective cohort study of Spanish adults found a significant association between low serum vitamin D levels and increased risk of incident obesity over 12 years of follow-up [52]. Our study of Norwegian adults aged 19-55 years at baseline showed similar results after a follow-up period of 11 years [27]. However, a study of Hispanic and African American adults from the United States did not find an association between serum vitamin D levels and BMI change over 5 years of follow-up [50]. Another study of elderly women did not find a significant association overall between 25(OH)D status and weight change over 4.5 years of follow-up. However, women with higher serum 25(OH)D concentration tended to gain less weight than women with lower serum 25(OH)D among a subgroup of women who gained weight during the follow-up [49].

Some evidence suggests that adults with normal body weight at baseline tend to experience greater weight gain than initially overweight/obese individuals during long-term follow-up [54]. Thus, the association between vitamin D and weight change may differ between baseline body weight groups. For example, there may be an inverse association between vitamin D and weight change in the baseline normal weight group, implying a protective role of vitamin D against obesity; alternatively, there may be no association in the baseline overweight/obese

group, implying no treatment effect of vitamin D on obesity. Randomized controlled trials (RCTs) have yielded inconsistent results on the effect of vitamin D in reduction of weight in already overweight/obese individuals [55-59].

Sex, age, and physical activity could be important modifiers of the relationship between low vitamin D status and weight gain. Overweight/obesity is more prevalent among females than males. It has been proposed that this observation may be due to biological factors associated with fat storage and reproduction [60, 61]. Research suggests that increased exposure to poverty and social inequality in female populations may also contribute to gender disparities in overweight/obesity, particularly in LMICs [62, 63]. Meanwhile, it is unclear if a consistent relationship exists between sex and vitamin D status; some studies have found female participants to have lower average levels of serum vitamin D [33, 64], while others have found vitamin D deficiency to be more prevalent among male participants [28, 65, 66]. BMI tends to increase throughout the lifespan until 50-59 years of age, followed by a decline after the age of 60 [67]. Vitamin D deficiency is more common in aging populations due to decreased vitamin D production in the skin [68] in addition to other physiological and behavioral factors [69]. Physical activity is known to reduce risk of overweight/obesity [70, 71], but it has also been shown to elevate serum vitamin D levels [72, 73].

1.2 Rationale

1.2.1 Rationale for study

Given the widespread nature and damaging health effects of obesity, there is a need reduce the burden of obesity in both HICs and LMICs. Obesity is a disorder of chronic positive energy balance, and it usually appears over a relatively long duration [74]. Thus, identifying risk

factors is the first step to prevent excessive weight gain and development of obesity. Low serum vitamin D status has wide-ranging health effects, including a possible link to weight gain over time. However, longitudinal studies of the relationship between vitamin D status and body weight have yielded inconsistent results. This relationship must be clarified in order to prevent obesity and promote health across the lifespan.

1.2.2 Research questions

Based on an extensive review of available literature on the topic, we established the following research questions:

1. Is higher serum 25(OH)D concentration associated with a lower weight change and a reduced risk of weight gain in normal weight adults over time?
2. If so, to what extent?

1.2.3 Objective & specific aims

The objective of this study was to investigate the relationship of serum 25(OH)D level with weight change and risk of weight gain in normal weight adults participating in the Nord-Trøndelag Health Study (HUNT) over an average of 11 years of follow-up. Specific aims were as follows:

1. to evaluate the association between 25(OH)D level and percentage annual weight change,
2. to evaluate the association between 25(OH)D level and risk of weight gain, and
3. to evaluate possible modification by sex, age, and physical activity on the association between 25(OH)D level and risk of weight gain.

1.2.4 Hypothesis

Based on the findings of previous studies, we hypothesize that higher serum 25(OH)D level is associated with lower percentage annual weight change and decreased risk of weight gain. We also hypothesize that the effect of 25(OH)D level on weight gain will differ at varying values of sex, age, and physical activity.

2. MATERIALS & METHODS

2.1 Study population & data collection

HUNT is a large and comprehensive health study in Norway; it has previously been described in detail [75, 76]. Data from the second and third surveys of HUNT, HUNT2 (1995-1997) and HUNT3 (2006-2008), were used for this study (see Appendix A). All residents in the Nord-Trøndelag region of Norway aged ≥19 years were invited to participate in each of the surveys. Data were collected via questionnaires and clinical examinations that were performed by trained health professionals [75]. Height and body weight were measured in both HUNT2 and HUNT3. Participants wore light clothing and no shoes during measurements, with height measured to the nearest 1.0 cm and weight to the nearest 0.5 kg. BMI was calculated as baseline

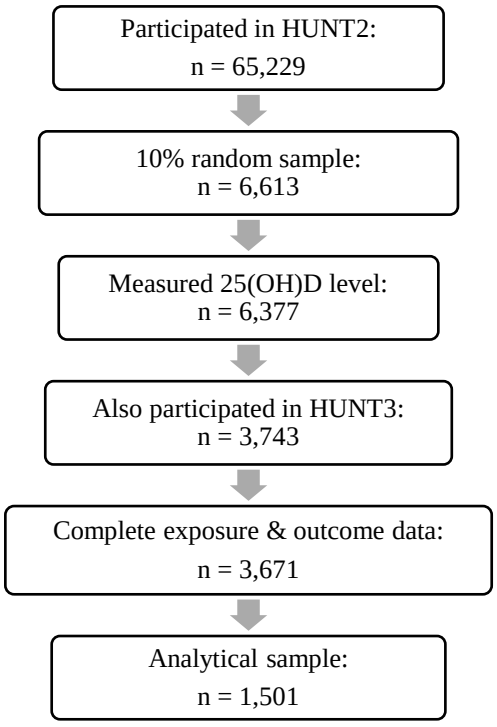

Figure 1. Selection of the study population and analytical sample, Nord-Trøndelag Health Study (HUNT). Analytical sample comprised of participants with normal weight (BMI ≥18.5 and <25 kg/m^2) at baseline and complete data on serum 25-hydroxyvitamin D level [25(OH)D], height, and body weight.

weight (kg) divided by height squared (m^2) at baseline. Of the approximately 93,000 individuals invited to participate in HUNT2, 65,229 took part in the study (response rate: 70%), and a 10% random sample (n=6,613) was selected for measurement of serum 25(OH)D levels. Baseline levels were determined for participants with sufficient blood sample volume (n=6,377), of which 3,671 participated in HUNT3 for an average of 11-year follow-up. Participants with incomplete data on serum 25(OH)D, height, or body weight were excluded from analysis. The final analytical sample was composed of individuals with normal weight at baseline (n=1,501) (Figure 1). Normal weight was defined as having a BMI of $\geq$18.5 kg/m^2 and <25 kg/m^2 using standard WHO cutoff-points [77]. Only normal weight individuals were included in analysis to prevent any possible therapeutic effect of serum 25(OH)D on body weight in overweight and obese individuals from obscuring study results.

2.3 Serum 25(OH)D level as exposure variable

Blood samples were collected from HUNT2 participants and stored in -20°C freezers at the HUNT Biobank (Levanger, Norway). LIAISON 25-OH Vitamin D TOTAL assay (DiaSorin, Saluggia, Italy) was used to determine baseline serum 25(OH)D levels. This fully automated chemiluminescent immunoassay has a detection range of 10-375 nmol/L. The assay has intra- and inter-assay coefficients of variation of 4% and 8%, respectively. Because seasonal fluctuations in 25(OH)D levels were expected due to the high-latitude geographical position of Norway (Figure 2a), a cosinor model based on month of blood draw was used to calculate season-standardized 25(OH)D level (nmol/L) that represents the annual average value of 25(OH)D for each subject [5]. The standardized 25(OH)D level was well-correlated with the measured 25(OH)D level (Figure 2b). This model was based on the 10% random sample of

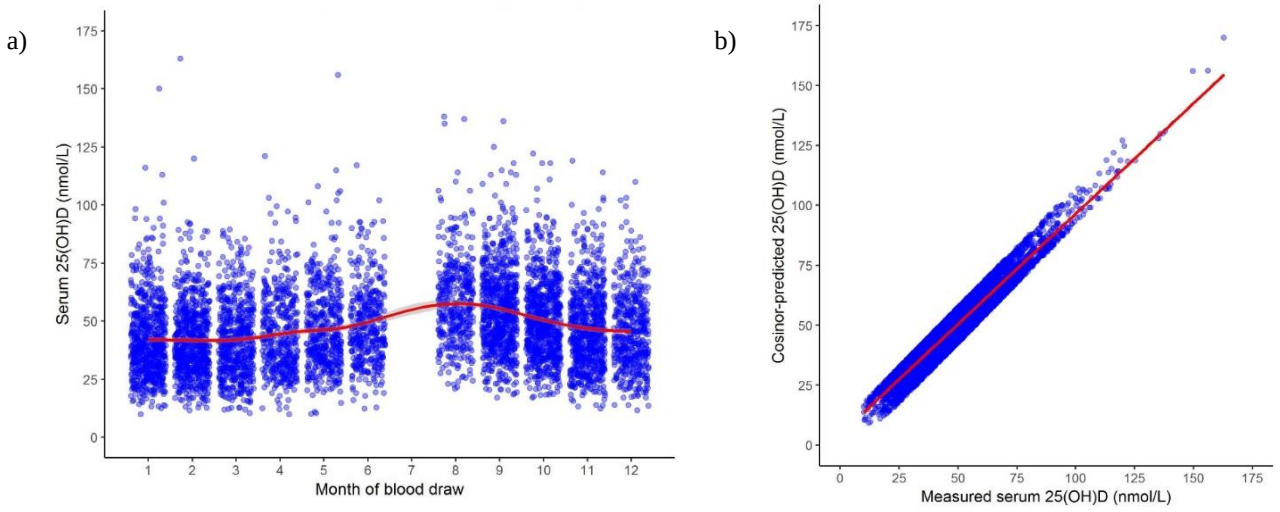

Figure 2. Serum 25-hydroxyvitamin D [25(OH)D] in the Nord-Trøndelag Health Study (HUNT) (n=6,377). a) Distribution of serum 25(OH)D levels (nmol/L) by month of blood draw (January-December). Serum 25(OH)D levels appear higher in summer months when sun exposure is greater than winter months in which sun exposure is lower. b) Comparison of measured serum 25(OH)D (nmol/L) and cosinor-predicted 25(OH)D (nmol/L). A linear relationship is evident.

HUNT 2 participants where serum 25(OH)D levels were measured for participants with sufficient blood sample volume (n=6,377) (Figure 1). It has been suggested that use of annual average 25(OH)D levels is a more effective method of controlling for season of blood draw than inclusion of season in adjusted models because it appears to minimize mean-squared error [78]. Mean squared error takes into account both bias squared and variance. The season-standardized 25(OH)D levels were treated as a categorical variable classified by the following cutoff-points for presentation of results (nmol/L): <25.0, 25.0-49.9, 50.0-74.9, and ≥75.0 (Table 1). These categories were established based on previous scientific literature [27, 79].

2.4 Covariates

Age, sex, smoking status, education, economic difficulties, alcohol consumption, physical activity, and sedentary lifestyle were identified as important covariates *a priori* due to possible confounding associations with exposure and outcome variables (Figure 3). All covariate

data were collected during HUNT2. Smoking status was categorized as never, former, and current smokers. Education was categorized as follows: 10, 10-12, and ≥13 years. Presence of economic difficulties was evaluated using the following question: "During the last year, has it at any time been difficult to meet the costs of food, transportation, housing and such?". Alcohol consumption was categorized as 0 (abstainer), 1-4, and ≥5 times/month. Physical activity level was classified into four categories: inactive, low, moderate, or high. Categorization of physical activity in the HUNT study has been described in detail previously [80]. Total sitting time was used as a marker for sedentary lifestyle and was categorized as follows (sitting hours/day): <4, 5-7, and ≥8. Separate "unknown" categories were established for participants missing information for the following covariates: smoking status (n=19), education (n=29), economic difficulties (n=257), alcohol consumption (n=95), physical activity (n=362), and sedentary lifestyle (n=272)

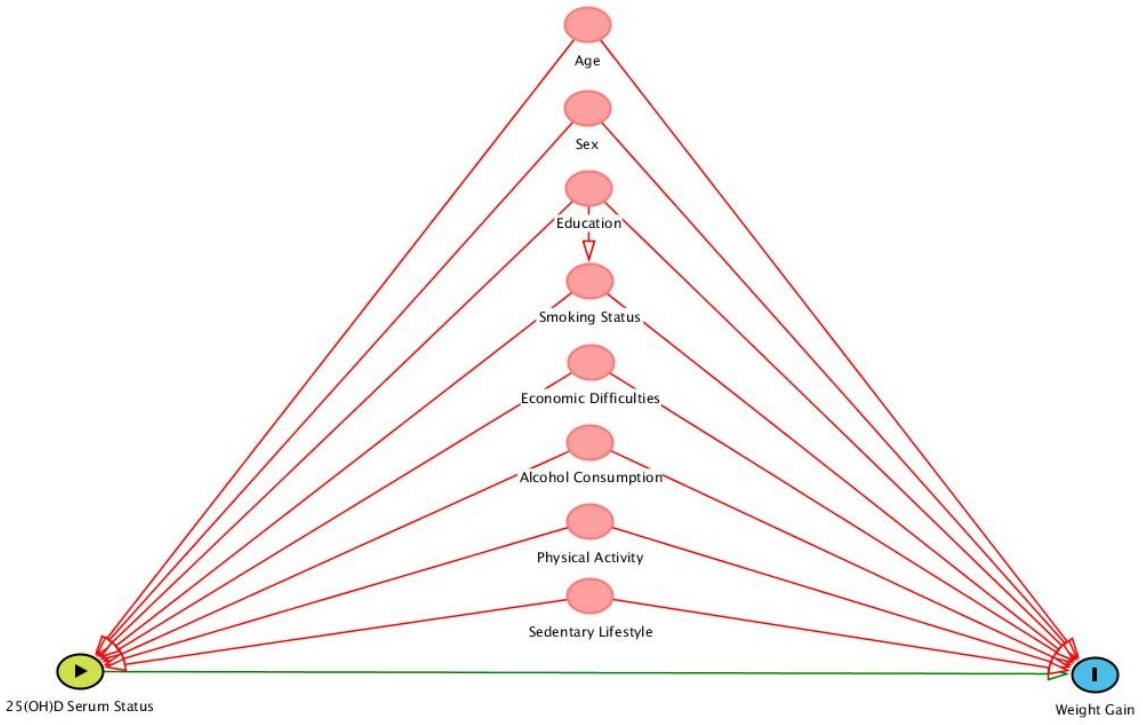

Figure 3. Directed acyclic graph (DAG) illustration of proposed relationships between exposure variable, outcome variable, and covariates.

(Table 2). "Unknown" categories were included in the primary analyses. These covariate categorizations have been used in previous HUNT publications [80-82]. A directed acyclic graph (DAG) describes the relationship between exposure and outcome with possible confounding by the covariates (Figure 3). We also note the possibility of complex relationships between covariates; however, this is beyond the scope of the present study.

2.5 Outcome variables

Percentage annual weight change (continuous variable) was calculated using participant weight change and follow-up time between HUNT2 and HUNT3. Annual weight gain (categorical variable yes/no) was defined as a percentage annual weight change of >1.25% based on cutoff-points used in previous studies [83]. Clinical weight gain (categorical variable yes/no) was defined as weight change of ≥5% over the 11-year follow-up. This cutoff-point was established in a previous publication after extensive consideration of biological relevance, expert

Table 1. Summary of exposure and outcome variables

Variable	Definition	Description
Baseline serum 25(OH)D level (nmol/L)	<25.0 25.0-49.9 50.0-74.9 ≥75.0	Independent variable (Exposure)
Percentage annual weight change* (continuous variable)	$\frac{(\text{HUNT3 weight-HUNT2 weight})/\text{HUNT2 weight}}{\text{follow up time (years)}} * 100$	Dependent variable (Outcome)
Annual weight gain (categorical variable)	Percentage annual weight change >1.25% between HUNT2 and HUNT3	Dependent variable (Outcome)
Clinical weight gain (categorical variable)	Weight change ≥5% between HUNT2 and HUNT3	Dependent variable (Outcome)

25(OH)D: 25-hydroxyvitamin D
*weight measured in kilograms; follow-up time calculated as years between HUNT2 and HUNT3 data collection

opinion, historical precedents, and applications within public health and clinical medicine [84]. It has been suggested that usage of both continuous and categorical outcome variables in evaluation of weight change allows for clear modelling of overall linear relationships and relevant directional information within exposure groups. This type of weight change modelling has also been suggested to increase comparability between studies [83].

2.6 Statistical analysis

Descriptive statistics were calculated for the analytical sample of included normal weight adults (n=1,501) and stratified by the categories of serum 25(OH)D level for the following demographic and explanatory variables: age, sex, smoking status, education, economic difficulties, alcohol consumption, physical activity, and sedentary lifestyle. All covariates were retained in final models due to plausible confounding relationships with exposure and outcome variables. Differences in distribution of baseline covariates between the 25(OH)D level categories were assessed using analysis of variance (ANOVA) for continuous variables and the Pearson chi-square tests for categorical variables.

Multivariable linear regression was used to evaluate the association between baseline 25(OH)D level and percentage annual weight change between HUNT2 and HUNT3; crude and adjusted coefficients and 95% confidence intervals (CI) were estimated. Multivariable logistic regression was used to investigate the relationship between baseline serum 25(OH)D categories and risk of annual weight gain and risk of clinical weight gain; crude and adjusted odds ratios (OR) and 95% CIs were estimated. The lowest serum 25(OH)D category (<25.0 nmol/L) was used as the referent group in both analyses. The linear trend across 25(OH)D levels was estimated when serum 25(OH)D was entered in the models as a continuous variable.

Effect modification by sex, age, and physical activity for clinical weight gain was evaluated using likelihood ratio test. Age and physical activity were categorized as binary variables with similar number of participants to maintain statistical power in the stratification analysis. Age was categorized as <45 and ≥45 years, and physical activity categories were collapsed into two groups: low (defined as inactive or low) and high (defined as moderate or high). All statistical analyses were conducted using Stata, release 14 (StataCorp LP, College Station, Texas).

2.7 Ethical considerations

Written informed consent was obtained prior to participation for all participants. The study was conducted as a sub-study under the approval of the Regional Committee for Medical Research Ethics (REK) (2015/1562 REK sør-øst C). Data was anonymized upon receipt via removal of personal identification numbers and names. All required information for the present analyses was available at the HUNT Research Center; therefore, no HUNT participants were contacted for additional data collection (see Appendix B).

3. RESULTS

3.1 Baseline characteristics of study population

The study population was predominately female compared to male (61.4% *vs.* 38.7%, respectively), with a lower proportion of women was in the highest serum 25(OH)D level group compared to the lowest level group (Table 2). More study participants had baseline serum 25(OH)D levels in the two mid-level categories (46.6% in 25.0-49.9 nmol/L and 41.2% in 50.0-74.9 nmol/L groups) than the lowest or highest categories (3.0% in <25.0 nmol/L and 8.6% in ≥75.0 nmol/L groups). Study participants were on average 43.3 years of age; mean age was approximately five years higher in the group with the highest serum 25(OH)D level compared to the lowest. Participants with higher baseline 25(OH)D levels (50.0-74.9 nmol/L or ≥75.0 nmol/L) tended to report the following characteristics in greater proportions than participants of lower baseline 25(OH)D levels (<25.0 nmol/L or 25-49.9 nmol/L): never smoking, ≥13 years of education, no economic difficulties, alcohol consumption 1-4 times/month, and moderate physical activity. The pattern was less clear for measures of sedentary lifestyle.

3.2 Serum 25(OH)D level & percentage annual weight change

A lower mean percentage annual weight change was present for all other 25(OH)D categories compared to the <25.0 nmol/L group (Table 3). However, this observation was not statistically significant in the adjusted model. Every 25 unit increase in serum 25(OH)D was associated with a 0.11% (95% CI -0.17– -0.05) reduction in percentage annual weight change in crude analysis, but the coefficient was attenuated to 0.05% with a wider 95% CI (-0.11–0.01) in the adjusted model.

3.3 Serum 25(OH)D, risk of annual weight gain, & risk of clinical weight gain

We evaluated the association between serum 25(OH)D level and risk of annual weight gain and of clinical weight gain, adjusting for the aforementioned covariates (Table 4). Overall, 201 participants experienced annual weight gain (13.4%) and 708 experienced clinical weight (47.2%). An inverse relationship was present between increasing 25(OH)D level and the proportion of individuals experiencing annual weight gain during follow-up (26.7% in the <25.0 nmol/L group, 14.9% in 25.0-49.9 nmol/L, 12.1% in 50.0-74.9 nmol/L, and 7.0% in ≥75 nmol/L). The proportion of individuals experiencing clinical weight gain over follow-up showed a similar trend (62.2% in the <25.0 nmol/L group, 51.5% in 25.0-49.9 nmol/L, 43.2% in 50.0-74.9 nmol/L, and 38.0% in ≥75 nmol/L). A decreased risk of both annual weight gain and clinical weight gain was observed in all groups compared to the <25 nmol/L group with a dose-response relationship. Adjusting for relevant covariates attenuated the results, but a significant association was still observed for the ≥75 nmol/L group (OR=0.32 for annual weight gain, OR=0.46 for clinical weight gain). Trends of decrease in risk of annual weight gain and clinical weight gain across 25(OH)D levels were also observed when 25(OH)D was evaluated as a continuous variable; risk of annual weight gain decreased by 23% per 25 nmol/L increase in 25(OH)D, and risk of clinical weight gain decreased by 20%.

3.4 Effect modification by sex, age, & physical activity

We also evaluated the association between serum 25(OH)D and clinical weight gain stratified by sex, age, and physical activity (Table 5). The proportion of individuals experiencing clinical weight gain over follow-up was higher for males and participants <45 years of age compared to females and participants ≥45 years of age (49.9% *vs.* 45.4% and 58.2% *vs.* 32.8%,

respectively). However, there was not a large difference between the proportions of individuals experiencing clinical weight gain over follow-up between those reporting low versus high physical activity (49.4% vs. 50.0%, respectively).

A significant reduction in risk of clinical weight gain per 25 nmol/L increase in serum 25(OH)D was observed for female participants (OR=0.70, 95% CI (0.57-0.87)), but no obvious association was observed for males (*p-value* for interaction=0.06). Risk of clinical weight gain significantly decreased by 35% per 25 nmol/L increase in serum 25(OH)D for subjects engaging in low levels of physical activity, but it was associated with a 9% non-significant reduction for those reporting high physical activity (*p-value* for interaction=0.07). A significant reduction in risk of clinical weight gain per 25 nmol/L increase in serum 25(OH)D was observed for participants aged <45 years (OR=0.78, 95% CI (0.63-0.97)). A similar risk reduction was observed for participants ≥45 years of age but with a wider confidence interval (OR 0.81, 95% CI (0.61-1.07)), and the *p-value* of likelihood ratio test for interaction between serum 25(OH)D and age was 0.84.

Table 2: Baseline characteristics of subjects overall and by baseline serum 25(OH)D levels in the HUNT2 study, 1995-1997

Characteristic	Overall N=1501	Baseline seasonal-standardized serum 25(OH)D level (nmol/L)				p^a
		<25.0 *n*=45	**25.0-49.9** *n*=699	**50.0-74.9** *n*=628	**≥75.0** *n*=129	
Age (years)	43.4±13.2	39.7±11.5	42.6±12.2	44.2±14.0	45.3±14.6	0.01
Sex						0.08
Male	579 (38.7%)	13 (28.9%)	273 (39.1%)	232 (36.9%)	61 (47.3%)	
Female	922 (61.4%)	32 (71.1%)	426 (60.9%)	396 (63.1%)	68 (52.7%)	
Smoking Status						<0.001
Never	689 (45.9%)	13 (28.9%)	306 (43.8%)	308 (49.0%)	62 (48.1%)	
Former	332 (22.1%)	10 (22.2%)	136 (19.5%)	150 (23.9%)	36 (27.9%)	
Current	461 (30.7%)	22 (48.9%)	246 (35.2%)	163 (26.0%)	30 (23.3%)	
Unknown	19 (1.3%)	0 (0.0%)	11 (1.6%)	7 (1.1%)	1 (0.8%)	
Education (years)						0.01
<10	377 (25.1%)	14 (31.1%)	192 (27.5%)	147 (23.4%)	24 (18.6%)	
10-12	522 (34.8%)	16 (35.6%)	238 (34.0%)	217 (34.6%)	51 (39.5%)	
≥13	573 (38.2%)	13 (28.9%)	248 (35.5%)	259 (41.2%)	53 (41.1%)	
Unknown	29 (1.9%)	2 (4.4%)	21 (3.0%)	5 (0.8%)	1 (0.8%)	
Economic difficulties						0.01
No	911 (60.7%)	20 (44.4%)	399 (57.1%)	406 (64.6%)	86 (66.7%)	
Yes	333 (22.2%)	13 (28.9%)	175 (25.0%)	123 (19.6%)	22 (17.1%)	
Unknown	257 (17.1%)	12 (26.7%)	125 (17.9%)	99 (15.8%)	21 (16.3%)	
Alcohol Consumption (times/month)						0.003
0 (Abstainer)	397 (26.5%)	19 (42.2%)	199 (28.5%)	153 (24.4%)	26 (20.2%)	
1-4	814 (54.2%)	16 (35.6%)	385 (55.1%)	345 (54.9%)	68 (52.7%)	
≥5	195 (13.0%)	5 (11.1%)	73 (10.4%)	89 (14.2%)	28 (21.7%)	
Unknown	95 (6.3%)	5 (11.1%)	42 (6.0%)	41 (6.5%)	7 (5.4%)	

Characteristic (cont).	**Overall** (cont.)	**Baseline seasonal-standardized serum 25(OH)D level (nmol/L)** (cont.)				***p*[a]** (cont.)
		<25.0	**25.0-49.9**	**50.0-74.9**	**≥75.0**	
Physical activity						0.003
Inactive	278 (18.5%)	10 (22.2%)	150 (21.5%)	104 (16.6%)	14 (10.9%)	
Low	289 (19.3%)	6 (13.3%)	135 (19.3%)	121 (19.3%)	27 (20.9%)	
Moderate	413 (27.5%)	10 (22.2%)	180 (25.8%)	185 (29.5%)	38 (29.5%)	
High	159 (10.6%)	2 (4.4%)	56 (8.0%)	81 (12.9%)	20 (15.5%)	
Unknown	362 (24.1%)	17 (37.8%)	178 (25.5%)	137 (21.8%)	30 (23.3%)	
Sedentary lifestyle (sitting hours/day)						0.05
<4	417 (27.8%)	7 (15.6%)	191 (27.3%)	179 (28.5%)	40 (31.0%)	
5-7	375 (25.0%)	13 (28.9%)	159 (22.7%)	172 (27.4%)	31 (24.0%)	
≥8	437 (29.1%)	12 (26.7%)	203 (29.0%)	182 (29.0%)	40 (31.0%)	
Unknown	272 (18.1%)	13 (28.9%)	146 (20.9%)	95 (15.1%)	18 (14.0%)	

25(OH)D: 25-hydroxyvitamin D; *HUNT2:* Nord-Trøndelag Health Study 2

Data are given as number of subjects (column percentage) or mean±standard deviation; Percentages (%) may not add up to 100% due to rounding

[a] Comparisons between baseline serum 25(OH)D level caterogies; p-values reported using Pearson chi-square tests for categorical covariates or ANOVA tests for continuous covariates

Table 3: Association between baseline seasonal-standardized serum 25(OH)D level and percentage annual weight change from 1995-97 to 2006-08

	No. of participants	Crude *coefficient* (95% CI)	Adjusted [a] *coefficient* (95% CI)
Seasonal-standardized serum 25(OH)D (nmol/L)			
Categorical			
<25.0	45	Reference	Reference
25.0-49.9	699	-0.21 (-0.46–0.03)	-0.14 (-0.37–0.10)
50.0-74.9	628	-0.32 (-0.56–-0.08)	-0.19 (-0.42–0.05)
≥75.0	129	-0.38 (-0.65–-0.11)	-0.22 (-0.49–0.04)
Continuous			
	1501	-0.11 (-0.17–-0.05)	-0.05 (-0.11–0.01)

25(OH)D: 25-hydroxyvitamin D; *CI*: confidence interval

[a] Adjusted for age, sex, smoking status, education, economic difficulties, alcohol consumption, physical activity, and sedentary lifestyle

For categorical 25(OH)D, the coefficient is the difference of percentage annual weight change compared with the reference group; for continuous 25(OH)D, the coefficient is the change in percentage annual weight change per 25 nmol/L increase in 25(OH)D.

Table 4: Association between baseline seasonal-standardized serum 25(OH)D level and annual weight gain and clinical weight gain from 1995-97 to 2006-08

	Annual weight gain				Clinical weight gain			
	Cases	**Risk**	**Crude OR (95% CI)**	**Adjusted OR (95% CI)***	**Cases**	**Risk**	**Crude OR (95% CI)**	**Adjusted OR (95% CI)***
Seasonal-standardized serum 25(OH)D (nmol/L)								
Categorical								
<25.0	12	26.7%	1.00	1.00	28	62.2%	1.00	1.00
25.0-49.9	104	14.9%	0.48 (0.24-0.96)	0.63 (0.30-1.31)	360	51.5%	0.64 (0.35-1.20)	0.71 (0.37-1.36)
50.0-74.9	76	12.1%	0.38 (0.19-0.76)	0.56 (0.27-1.20)	271	43.2%	0.46 (0.25-0.86)	0.56 (0.29-1.07)
≥75.0	9	7.0%	0.21 (0.08-0.53)	0.32 (0.12-0.87)	49	38.0%	0.37 (0.18-0.75)	0.46 (0.22-0.97)
Continuous								
			0.66 (0.52-0.83)	0.77 (0.60-0.99)			0.73 (0.63-0.86)	0.80 (0.68-0.95)

25(OH)D: 25-hydroxyvitamin D; *OR:* odds ratio; *CI:* confidence interval

Cases are given as number of subjects (percentage); Percentages (%) may not add up to 100% due to rounding

*Adjusted for age, sex, smoking status, education, economic difficulties, alcohol consumption, physical activity, and sedentary lifestyle

Annual weight gain was defined as percentage annual weight change >1.25% between HUNT 2 and HUNT 3.

Clinical weight gain was defined as weight change ≥5% between HUNT 2 and HUNT 3.

Table 5: Association between baseline seasonal-standardized serum 25(OH)D level and clinical weight gain over 11-year follow-up, stratified by sex, age, and physical activity

	No. of participants	Cases	Risk	Adjusted OR (95% CI)*	p-value for interaction
Sex					
Male	579 (38.7%)	289	49.9%	1.00 (0.75-1.32)	0.06
Female	922 (61.4%)	419	45.4%	0.70 (0.57-0.87)	
Age (years)					
<45	851 (56.7%)	495	58.2%	0.78 (0.63-0.97)	0.84
≥45	650 (43.3%)	213	32.8%	0.81 (0.61-1.07)	
Physical activity					
Low	567 (49.8%)	280	49.4%	0.65 (0.49-0.87)	0.07
High	572 (50.2%)	286	50.0%	0.91 (0.69-1.20)	

25(OH)D: 25-hydroxyvitamin D; *OR:* odds ratio; *CI:* confidence interval

Data are given as number of subjects (column percentage); Percentages (%) may not add up to 100% due to rounding

*per 25 nmol/L increase in 25(OH)D; Adjusted for age, sex, smoking status, education, economic difficulties, alcohol consumption, physical activity, and sedentary lifestyle

Clinical weight gain was defined as weight change ≥5% between HUNT 2 and HUNT 3.

Low physical activity was defined as physical activity of inactive or low; high physical activity was defined as physical activity of moderate or high

4. DISCUSSION

4.1 Main findings

This study sought to evaluate the effect of serum 25(OH)D concentration on three measures of weight change in a sample of 1,501 adults over an average of 11 years of follow-up: percentage annual weight change, risk of annual weight gain, and risk of clinical weight gain. A reduction in percentage annual weight change was present although not statistically significant as 25(OH)D increased by 25-unit increments. A dose-response relationship was observed between 25(OH)D level and risk of both annual weight gain and risk of clinical weight gain; in the 75 nmol/L group, risk of both outcomes was significantly reduced by 68% and 54% compared to the <25 nmol/L group, respectively. Our results were suggestive of effect modification by sex and physical activity on the association between 25(OH)D and risk of clinical weight gain, though significant *p-values* for interaction were not observed. Effect modification by age did not appear to be present.

4.2 Comparison to past literature

4.2.1 Vitamin D status as an independent determinant of weight gain

Several population-based studies have investigated the longitudinal association of serum vitamin D level with weight change [27, 49, 50, 52, 53, 85]. Our results were consistent with the findings from a previous HUNT study in which low 25(OH)D levels were associated with an increased risk of general and central obesity in young and middle-aged adults [27]. Similarly, another prospective cohort study found that individuals with baseline serum 25(OH)D levels of

≤42.5 nmol/L had >2-times the risk of developing obesity or experiencing weight increase >3.7 kg over four years of follow-up [52]. Our findings are also consistent with those from a prospective cohort study of 479 Colombian children that found significant associations between 25(OH)D levels of <50 nmol/L and yearly increases in BMI and waist circumference over three years [53]. Contrastingly, Young et al. found no significant evidence of associations between 25(OH)D and changes in BMI or measures of adipose tissue in a population of Hispanic and African-American adults after five years of follow-up [50]. However, the analysis adjusted for only age, gender, and adiposity phenotype, leaving a large possibility for residual confounding by lifestyle factors. Additionally, difference in follow-up duration may also contribute to the discrepancy between our findings and those of the aforementioned study. Although another longitudinal study of 4,659 elderly women did not find a significant association between 25(OH)D and weight change overall, higher baseline 25(OH)D level was significantly associated with less weight gain over 4.5 years of follow-up in the subgroup of women who gained weight [49]. Women in this subgroup were predominately of normal weight at baseline. This study also had a relatively shorter follow-up period than our study. In a recent analysis of three Northern European cohorts by Larsen et al., there was no evidence of a significant association between 25(OH)D level and annual changes in body weight or waist circumference over follow-up [85]. It is possible that a categorical measure of weight change could have been more effective in detecting an association than the actual continuous measure of weight change in terms of statistical power. For example, we also did not find a significant association between 25(OH)D level and percentage annual weight change but did find a significant association between 25(OH)D level and risk of both annual weight gain and clinical weight gain.

It is important to note that some variation between the results of the above-described observational studies and our study could be partially explained by differences in choice and categorization of exposure and outcome variables. Two studies evaluated serum 25(OH)D level as a binary variable (<30 ng/mL *vs.* ≥30 ng/mL and ≤17 ng/mL *vs.* >17 ng/mL) [49, 52], while others evaluated three levels of 25(OH)D status (<50, 50.0-74.9, and ≥75 nmol/L) [27, 53]. Two other studies evaluated serum 25(OH)D solely as a continuous variable [50, 85]. We categorized serum 25(OH)D exposure into four levels based on previous scientific literature to account for more variation in baseline vitamin D status in addition to evaluating it as a continuous variable. A great deal of variation was also present in evaluation of weight change over follow-up. Common approaches were evaluation of incident obesity (defined based on BMI or waist circumference) [27, 52], change in BMI [50, 53], change in waist circumference (overall or annual) [53, 85], change in body weight (overall, annual, percent, or various categorizations) [49, 52, 85], and alternative measures of adiposity like skinfold thickness [53, 65]. We used percentage annual weight change and percentage overall weight change to generate two categorical outcomes such as annual weight gain and clinical weight gain. Variation in the type of assay used to determine 25(OH)D concentration could also has some impact on comparability of our results with these studies, though this effect is likely to be minimal based on previous research into the issue [86].

While our results might be extended to infer that vitamin D supplementation might be associated with a reduced risk of weight gain over time in normal weight individuals, intervention and experimental trials utilizing vitamin D supplementation have yielded inconsistent results [55-59, 87]. However, these trials have been mostly conducted in overweight or obese individuals. Additionally, the intervention duration was usually short, ranging from six

weeks to one year. Clinical trials with vitamin D supplementation in overweight or obese individuals may be useful in examining the possible therapeutic effect rather than the preventive effect of vitamin D on weight change. Only one large RCT showed that participants of normal weight who received a daily supplement of 1000 mg of calcium and 400 IU of vitamin D experienced significantly lower average annual weight gain and lower risk of weight gain over seven years of follow-up compared to a placebo group [87]. However, it is impossible to isolate the effects of calcium versus vitamin D on weight change in this study due to the use of a combined supplement.

4.2.2 Effect modification by sex, age, & physical activity

We evaluated sex, age, and physical activity as potential effect modifiers of the association between serum 25(OH)D and clinical weight gain. To our knowledge, this is one of the first prospective cohort studies of its kind to extensively evaluate effect modification with the given exposure and outcome variables.

Our data indicate possible effect modification by sex. Among female participants, we found a significant 30% reduction in risk of clinical weight gain per 25 nmol/L increase in serum 25(OH)D. No significant association was found in the male subgroup. These findings indicate that vitamin D status could be a more important factor in clinically significant weight gain for women than men. There is some evidence to suggest that the effect of vitamin D supplementation on cardiometabolic biomarkers varies by sex, though results from this study are unlikely to be generalizable since it was performed in a small sample of patients with non-alcoholic fatty liver disease (n=53) [88]. Although population studies of the relationship between vitamin D status and sex have reported mixed results [28, 33, 64-66], pregnant women and

women of child-bearing age are thought to be at increased risk for vitamin D deficiency [12, 31]. Indeed, the proportion of women in the 25(OH)D <25 nmol/L group appeared higher than those in the other vitamin D categories in the present study. Additionally, obesity is more prevalent in female than male populations, due in part to biological factors [60-63]. One other prospective cohort study has examined sex as a possible effect modifier for serum 25(OH)D and weight change [53]. This study did not find effect modification by sex in examinations of change in BMI or change in waist circumference. However, follow-up time was relatively short (3 years), the sample size was smaller than ours (n=479), and the authors did not report specific results from the stratified analysis.

Age did not appear to be an effect modifier. Although this finding is somewhat counterintuitive since both vitamin D deficiency and obesity are typically more prevalent in older than younger populations [67-69], it can likely be explained by factors unique to this study population as individuals with 25(OH)D ≥75 nmol/L were generally older compared with those with vitamin D <25 nmol/L. Supplemental cod liver oil is a significant source of vitamin D in the Nordic diet [89], and research from the HUNT population suggests that older age tends to correspond with increased supplementation [82]. Our findings suggest that vitamin D status may be an important determinant of weight gain in both younger and older adults, but we should be cautious to generalize this finding to populations outside of the Nordic region.

Results were also indicative of potential effect modification by physical activity. Participants engaging in low physical activity were found to have a significant 35% reduction in risk of clinical weight gain per 25 nmol/L increase in serum 25(OH)D, while results for highly active participants were much weaker. Though not exactly comparable due to differences in study design, these results are consistent with the findings of the previously discussed RCT on

vitamin D supplementation [87]. Over follow-up, participants in the lowest physical activity group taking the vitamin D supplement experienced significantly smaller average annual weight gain than women taking the placebo. Results for women in the two higher activity groups did not differ significantly from the placebo group. Since physical activity is an important factor for prevention of weight gain and has been shown to elevate serum 25(OH)D [70-73], our findings and the findings of the vitamin D supplementation trial suggest that high levels of physical activity might mask the effect of serum 25(OH)D on weight change over time. However, the role of vitamin D as a preventative factor for weight gain may be particularly important for individuals engaging in low levels of physical activity due to absence of the protective effect of high activity.

4.3 Biological plausibility

The exact mechanism by which serum 25(OH)D might affect changes in body weight has not been well-established. However, several studies have proposed plausible pathways by which this might occur. Adipose tissue is the main site for vitamin D storage [90], but it has also been found to express vitamin D receptors [91] and vitamin D-metabolizing enzymes [92]. In vitro studies have suggested that vitamin D plays a regulatory role in adipocytes by promoting lipogenesis and inhibiting lipolysis [93, 94]. Additionally, studies have suggested that vitamin D may influence adipocyte apoptosis [95-97]. In mouse models, increased dietary vitamin D intake has been shown to trigger weight reduction via adipocyte apoptosis [95]. In vitro, studies suggest that high doses of vitamin D induce a calcium-mediated signaling cascade that leads to adipocyte apoptosis; conversely, low doses of vitamin D have been shown to inhibit apoptosis through suppression of the primary enzymes in this cascade [96, 97].

Vitamin D may also function in energy homeostasis via regulation of leptin and adiponectin expression [40]. Leptin is a hormone secreted by adipocytes following a meal that signals a high energy state to the hypothalamus, thereby promoting satiety, inhibiting hunger, and increasing energy expenditure [98, 99]. Experimental evidence suggests that leptin also inhibits insulin-dependent glucose uptake by adipocytes [100] in addition to promoting lipolysis [101] and inhibiting lipogenesis [102]. Recent studies have indicated that vitamin D acts at a transcriptional level to upregulate mRNA expression and promote leptin secretion [103, 104]. Adiponectin is an adipocyte hormone that functions in inflammation reduction and enhancement of insulin sensitivity [105]. Low circulating adiponectin has been associated with several characteristics of metabolic syndrome, including increased visceral adiposity [106], insulin resistance [106, 107], high plasma triglycerides, elevated low-density lipoprotein cholesterol, and diminished high-density lipoprotein cholesterol [107, 108]. Consumption of vitamin D fortified foods has been shown to increase serum adiponectin levels [109], and vitamin D has also been shown to increase adiponectin levels in vitro [110].

4.4 Strengths & limitations

To our knowledge, this prospective cohort study is the first to investigate the relationship between serum 25(OH)D and weight change using outcomes such as risk of annual weight gain and risk of clinical weight gain. Additionally, the sample size was sufficient to perform a stratified analysis by sex, age, and physical activity. Evaluation of physical activity as a possible effect modifier of the association between serum 25(OH)D and weight change has not been included in similar analyses. Inclusion of comprehensive information on relevant covariates minimized confounding in adjusted models and provided more accurate estimates of coefficients

and odds ratios. Use of seasonal-standardized serum 25(OH)D as opposed to adjustment for season of blood draw allowed for minimization of mean-squared error, as discussed previously. Furthermore, the 11-year follow-up period allowed us to evaluate long-term weight change in the study population.

There were several limitations related to our study. Participation rates decreased from HUNT2 (71%) to HUNT3 (54%), increasing the possibility of selection bias [111]. In the stratified analysis, serum 25(OH)D could only be evaluated as a continuous variable due to concerns about statistical power. Additionally, evaluation of physical activity as an effect modifier could only be performed in 1,139 of the 1,501 subjects due to missing data on baseline physical activity. Thus, our findings on the effect modification by sex, age, and physical activity should be confirmed by studies with a larger sample size. Since data on lifestyle factors were self-reported, it is possible that data could be subject to misclassification. Our analysis did not account for changes in seasonal-standardized serum 25(OH)D level or lifestyle that may have taken place over the course of follow-up since these data were collected at baseline. Although the independent association between serum 25(OH)D level and weight change remained in the ≥75.0 nmol/L group after adjustment for relevant covariates, it is possible that residual confounding was present due to unknown or unmeasured factors. Information on dietary factors was not collected in the HUNT2 questionnaire, which may be significant since diets high in red meat and low in fruits and vegetables have been linked to increased risk of weight gain or obesity [112, 113]. However, socioeconomic status can reasonably be used as a proxy variable for dietary factors due to the observation that diet quality tends to vary based on socioeconomic status [114]. Additionally, the measure of physical activity may be prone to bias since type of leisure-time activity was not specified at data collection, though the present method of classification has been

considered sufficient [115]. Missing data on some baseline characteristics were regarded as a separate “unknown” category, which may have also resulted in residual confounding. Despite these limitations, this study provides a contribution to our understanding of the influence of serum 25(OH)D on weight change over time in normal weight adults.

4.5 Public health implications & future research

Taken in combination with widely accepted evidence that obesity can lead to low vitamin D status [40-43], our results suggest that a bidirectional association may exist in which low vitamin D status increases the risk of weight gain and obesity amplifies the risk of low vitamin D status. While this cycle could stand as an additional challenge in obesity prevention and treatment efforts, it also offers a new set of opportunities since low vitamin D status is a relatively easily modifiable risk factor. Therefore, interventions addressing vitamin D deficiency could be expected to somewhat influence obesity incidence, and vice versa. For example, public health efforts like vitamin D supplementation and food fortification have been established as viable methods for addressing low vitamin D status. Our findings indicate that these efforts could also be preventative measures in combatting the global obesity epidemic. This holds significance for HICs that serve as strongholds of the obesity epidemic but may be of particular importance for LMICs facing “double burdens” of disease where obesity is increasing in prevalence while rates of malnutrition remain high. The link between vitamin D deficiency and obesity should be taken into account by policymakers, clinicians, and public health professionals as novel approaches emerge for obesity prevention and treatment. At-risk groups for low vitamin D status like pregnant women, non-Western immigrants, children, elderly individuals, and women living in the Middle East should also be identified for targeted preventative measures.

Given the widespread nature and wide-ranging health effects of both vitamin D deficiency and obesity, additional research is needed to clarify the relationship between vitamin D status, weight change, and relevant covariates like physical activity and sex. Further experimental studies are also warranted in order to increase understanding of the underlying mechanisms by which vitamin D influences body weight. Our study was performed in normal weight Norwegian adults, which could limit the generalizability of our results to already overweight or obese adults. The effect of vitamin D supplementation on obese individuals warrants further investigation. Additionally, the present analyses should be replicated in a large, prospective cohort with more complete information on lifestyle factors like diet. Since vitamin D deficiency and obesity exist in a variety of contexts globally, it is important to also explore their relationship within the context of LMICs and other groups at-risk for vitamin D deficiency.

5. CONCLUSION

In summary, data from our prospective cohort of normal weight Norwegian adults provide evidence that high serum 25(OH)D level is associated with reduced risk of both annual weight gain and clinical weight gain. Additionally, our results suggest that the effect of 25(OH)D level on weight change varies by sex and physical activity but not by age. The data indicate that adequate vitamin D status may be particularly important in preventing weight gain for women or for individuals with low physical activity. Since obesity tends to be more prevalent in female populations and low physical activity is an established risk factor for weight gain, these findings also have broader public health implications in terms of targeted interventions. A thorough understanding of the relationship between vitamin D status and body weight is essential in order to prevent obesity, prolong life, and promote health in HICs and LMICs alike.

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APPENDIX A: HUNT Contract

NTNU
Fakultet for medisin og helsevitenskap
Institutt for samfunnsmedisin og sykepleie

Vår dato 03.04.2018
Vår referanse 2018/8828/TRS
Deres dato 16.03.2018
Deres referanse

Avtale

mellom
HUNT forskningssenter, MH, NTNU
og
Institutt for samfunnsmedisin og sykepleie, MH, NTNU

om bruk av forskningsdata fra Helseundersøkelsene i Nord-Trøndelag (HUNT) til masteroppgave for Adaline Heitz

Prosjekttittel: "Serum vitamin D levels in relation to change in body weight and waist circumference in Norwegian adults"

Avtalen bygger på prosjektbeskrivelse med publikasjonsplan datert 16.3.18. Avtalen bygger også på godkjenning i Regional komite for medisinsk og helsefaglig forskningsetikk (2015/1562/REK sør-øst C, datert 7.3.18).

Rammene for rettigheter til å analysere på HUNT-data er beskrevet i *Retningslinjer for forvaltning og bruk av data og biologisk materiale fra Helseundersøkelsene i Nord-Trøndelag, datert 14.3.16.* Prosjektleder er ansvarlig for at analysearbeidet skjer i henhold til disse retningslinjene. Prosjektleder har ansvar for datasikkerheten og at data oppbevares forsvarlig i henhold til lover og forskrifter.

En avidentifisert datafil er tidligere utlevert til prosjektet. Prosjektleder kan la andre personer få analysere på datafilen, så fremt arbeidet holder seg innenfor rammen for prosjektbeskrivelsen og publikasjonsplanen.

For å sikre at bruk av data skjer i samsvar med tildelte analyserettigheter skal, i henhold til gjeldende retningslinjer, alle manuskripter før innsending til publisering, forelegges publikasjonsutvalget ved HUNT forskningssenter i Levanger.

Når analysearbeidet er fullført og prosjektet avsluttes ønsker HUNT forskningssenter en dialog om hvilke data som skal tilbakeføres til HUNT databasen og hvordan slik tilbakeføring kan skje. Deretter skal datasettet slettes og bekreftelse på dette sendes skriftlig til HUNT forskningssenter,

Postadresse
Forskningsveien 2
7600 LEVANGER

Org.nr. 974 767 880
E-post:
hunt@medisin.ntnu.no
http://www.ntnu.no

Besøksadresse
Forskningsveien 2, Levanger

Telefon
+47 74 07 51 80

Saksbehandler
Turid Rygg Stene
Tlf: +47 74 07 51 98

Adresser korrespondanse til saksbehandlende enhet. Husk å oppgi referanse.

Levanger, jfr. punkt 11 i gjeldende retningslinjer. Dette skal ikke skje senere enn 31.12.19, med mindre ny avtale om forlengelse er inngått med HUNT forskningssenter.

Institutt for samfunnsmedisin og sykepleie, MH, NTNU

Trondheim, 20-04-2018

Sted og dato

Adaline Heitz

student Adaline Heitz

Xiao-Mei Mai

prosjektleder Xiao-Mei Mai

HUNT forskningssenter, MH, NTNU

Levanger, 3.4.18

Steinar Krokstad
professor dr. med./daglig leder

NTNU
Fakultet for medisin og helsevitenskap
Institutt for samfunnsmedisin og sykepleie

Vår dato 03.04.2018
Vår referanse 2018/8828/TRS
Deres dato 16.03.2018
Deres referanse

Xiao-Mei Mai
Institutt for samfunnsmedisin og sykepleie
Boks 8905
7491 Trondheim

Rettigheter til å analysere data fra Helseundersøkelsene i Nord-Trøndelag (HUNT)

Det vises til søknad vedrørende prosjektet «Serum vitamin D levels in relation to change in body weight and waist circumference in Norwegian adults» datert 16.3.18. Det vises også til søknad til og godkjenning fra Regional komite for medisinsk og helsefaglig forskningsetikk (2015/1562/REK sør-øst C, datert 7.3.18).

Søknaden er vurdert ved Forvaltningsgruppen ved HUNT forskningssenter. Prosjektet er interessant og kommer ikke i konflikt med andre pågående prosjekter i HUNT. Det gis med dette tilgang til bruk av aktuelle data og avtale om dette følger vedlagt. Vi ber om at avtalen undertegnes og returneres til HUNT forskningssenter, Levanger.

Avtalen gjelder for masterprosjekt for Adaline Heitz.

Kostnader for analyserettigheter til masteroppgave er kr. 2 000,-. Faktura sendes i eget brev.

Dersom det planlegges å benytte det utleverte datasettet til flere eller andre publikasjoner ut over det som er beskrevet i søknaden, forutsettes det at ny søknad med utfyllende publikasjonsplan oversendes til HUNT forskningssenter for ny vurdering.

Vi ønsker lykke til med forskningsarbeidet.

Med hilsen

Steinar Krokstad
professor dr. med./daglig leder

Inger D. Holbø
seniorkonsulent

Vedlegg: Avtale til undertegning
«Retningslinjer for forvaltning og bruk av data og biologisk materiale fra Helseundersøkelsene i Nord-Trøndelag» og «Retningslinjer for publisering av forskningsresultater som bruker HUNT-data», se http://www.ntnu.no/hunt/datatilgang

Postadresse Forskningsveien 2, 7600 LEVANGER
Org.nr. 974 767 880 E-post: hunt@medisin.ntnu.no http://www.ntnu.no
Besøksadresse Forskningsveien 2, Levanger
Telefon +47 74 07 51 80
Saksbehandler Turid Rygg Stene Tlf: +47 74 07 51 98
Adresser korrespondanse til saksbehandlende enhet. Husk å oppgi referanse.

APPENDIX B: REK Approval

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK sør-øst	Knut W. Ruyter	22845518	07.03.2018	2015/1562/REK sør-øst C
			Deres dato:	Deres referanse:
			11.01.2018	

Vår referanse må oppgis ved alle henvendelser

Xiao-Mei Mai
Institutt for samfunnsmedisin og sykepleie
NTNU

2015/1562 Genetisk og kausal påvirkning av vitamin D på risiko for hjerte- og karsykdommer

Forskningsansvarlig: Norges teknisk-naturvitenskapelige universitet
Prosjektleder: Xiao-Mei Mai

Vi viser til søknad om prosjektendring datert 11.01.2018 for ovennevnte forskningsprosjekt. Søknaden er behandlet av sekretariatet for REK sør-øst på fullmakt, med hjemmel i helseforskningsloven § 11.

Vurdering
I denne endringen søkes det om å utvide prosjektet til å studere om vitamin D nivåer også kan ha genetisk og kausal påvirkning på risiko for fedme og diabetes. Utvidelsen er beskrevet i en ny protokoll, og selve analysene skal utføres av 2 mastergradsstudenter som da også blir nye medarbeidere i prosjektet. Den ene er innmeldt i denne søknaden.

I henhold til protokoll skal kun allerede innsamlet materiale/opplysninger/analyser fra prosjektet fra HUNT benyttes for å studere sammenhengene mellom vitamin D mangel og risiko for diabetes og fedme. Vi legger til grunn at samtykke til HUNT også er dekkende for bruken av opplysningene til dette underprosjektet.

Komiteen anser underprosjektet som forenlig med det opprinnelige prosjektet og at det både er nyttig og viktig å undersøke sammenhenger som ennå ikke er gjort til gjenstand for forskning.

Underprosjektet blir i sin helhet godkjent. Når den andre mastergradsstudenten er på plass i 2019 for å undersøke sammenhengen med diabetes, kan det gjøres ved å melde inn ny medarbeider på skjema for prosjektendring.

Vedtak

Komiteen har vurdert endringsmeldingen og godkjenner prosjektet slik det nå foreligger med hjemmel i helseforskningsloven § 11.

Godkjenningen er gitt under forutsetning av at prosjektet gjennomføres slik det er beskrevet i endringsmeldingen og i protokollen "Vitamin D, physical activity, and cardio-metabolic disorders among adults in Norway".

Klageadgang

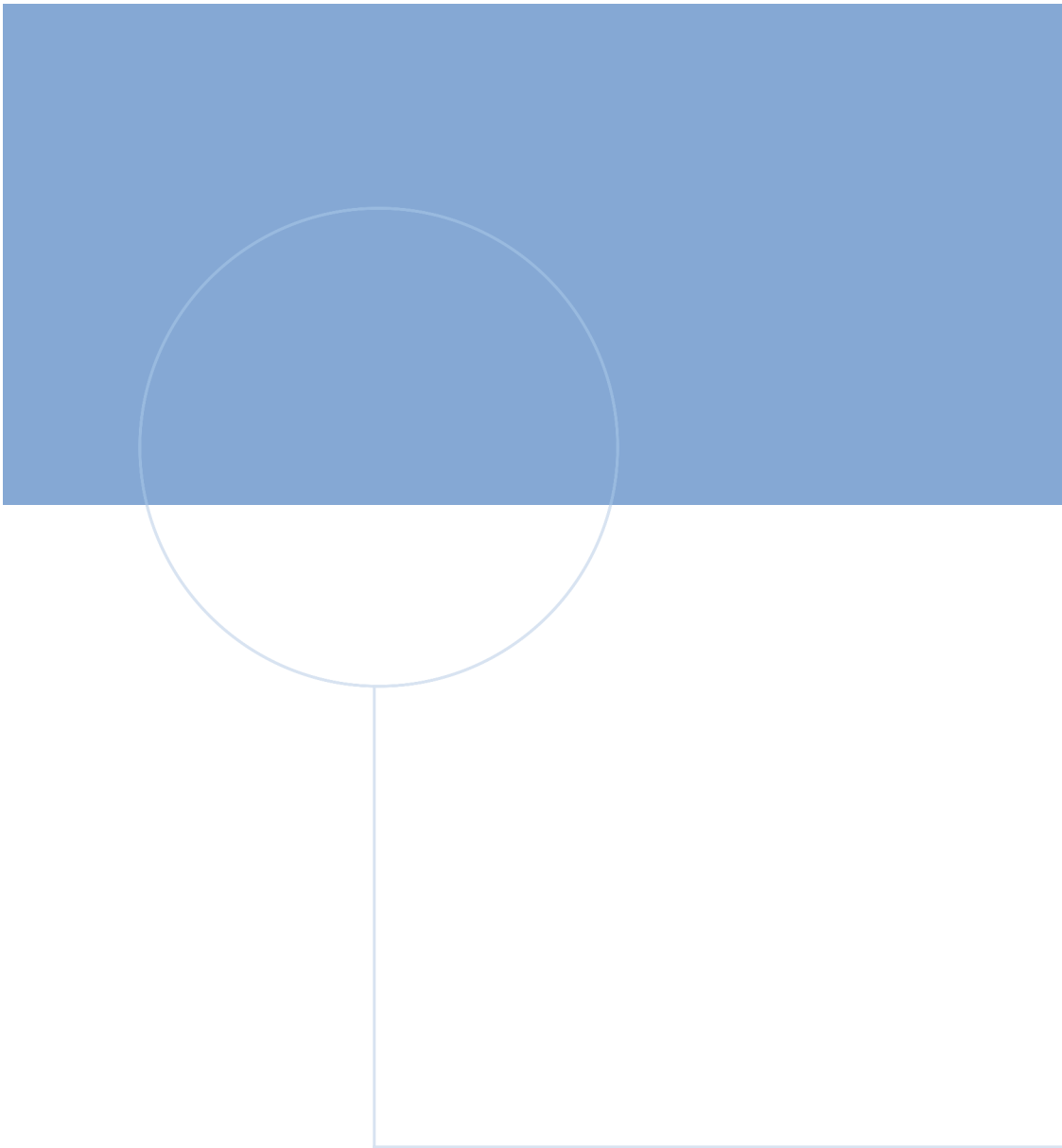
Besøksadresse: Gullhaugveien 1-3, 0484 Oslo
Telefon: 22845511
E-post: post@helseforskning.etikkom.no
Web: http://helseforskning.etikkom.no/

Du kan klage på komiteens vedtak, jf. helseforskningsloven § 10 og forvaltningsloven § 28 flg. Klagen sendes til REK sør-øst. Klagefristen er tre uker fra du mottar dette brevet. Dersom vedtaket opprettholdes av REK sør-øst, sendes klagen videre til Den nasjonale forskningsetiske komité for medisin og helsefag for endelig vurdering.

Med vennlig hilsen

Knut W. Ruyter
Avdelingsdirektør

Kopi til: *siri.forsmo@ntnu.no ; rek-ism@medisin.ntnu.no*



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