Vitamin D status among female handball and football elite athletes in Norway at latitude 60°N

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Abstract

Background: An unexpectedly high prevalence of vitamin D insufficiency has recently been reported worldwide, but little is known about the level in athletes. Vitamin D has known skeletal functions with high impact on bone health. The vitamin also has muscle and immune functions and low levels have been linked to decreased muscle strength in elderly and chronic diseases. If these functions can affect an athlete's health status and performance are yet to be established. **Purpose:** To measure serum vitamin D concentrations, as 25-hydroxyvitamin D (25(OH)D), in female elite athletes in handball and football in the south eastern parts of Norway, at latitude 60ºN. We also wanted to address possible exposure factors of the 25(OH)D level in this group, by investigating dietary intake, supplement use and sun behavior. **Methods:** Serum 25(OH)D concentrations were measured in 48 female elite athletes (26 handball/22 football) in October/November 2010. Insufficiency was defined as serum 25(OH)D concentration <80 nmol/l. Dietary intake was assessed with four-day weighed diet record. Supplement use and sun exposure were assessed via interviewer-assessed questionnaires. **Results:** Mean serum 25(OH)D level was 99 (SD=32) nmol/l. Vitamin D insufficiency was found in 27 percent of the subjects. Serum 25(OH)D levels were found to be significantly higher for the handball compared to the football players (p=0.001). The subjects residing only in Nordic countries in summer had a significantly lower 25(OH)D level than the subjects going outside of Nordic countries (p=0.039). The handball players resided significantly less in Nordic countries in summer than the football players (p=0.004). Seventy-three percent of all subjects had a vitamin D intake below the recommended intake of 7.5 µg/day. **Conclusion:** In this study, of female elite athletes living at high latitude, the average 25(OH)D level in fall was similar to optimal level, and vitamin D insufficiency was found in one out of four of the athletes. Going outside of Nordic countries in summer seemed to be the factor of most influence on the 25(OH)D levels. Since the subjects live at high latitude and since vitamin D intake was below recommendations for ¾ of the subjects, their 25(OH)D level is likely to decrease during winter. Hence, it needs to be debated if routine screening for 25(OH)D levels in female elite athletes should be recommended.

Norsk sammendrag

Bakgrunn: En overhyppighet av suboptimal vitamin D-status er blitt rapportert på verdensbasis, men lite er kjent når det gjelder nivået hos idrettsutøvere. Vitamin D er kjent for sin funksjon i skjelettet og betydning for beinhelse. I tillegg har vitamin D en funksjon i muskel og immunforsvaret, og lave nivåer er trolig forbundet med både nedsatt muskelstyrke og kroniske sykdommer. Om disse funksjonene kan påvirke helsestatus og prestasjon hos idrettsutøvere er ennå ikke bekreftet. **Hensikt:** Å måle serum vitamin D-konsentrasjon, som 25-hydroksyvitamin D (25(OH)D), hos kvinnelige toppidrettsutøvere innen håndball og fotball på Østlandet i Norge, ved breddegrad 60ºN. Vi ville også se på mulige eksponeringsfaktorer for 25(OH)D-nivået i denne gruppen, ved å se på kosthold, bruk av kosttilskudd og solvaner. **Metoder:** Serum 25(OH)D-konsentrasjon ble målt for 48 kvinnelige toppidrettsutøvere (26 håndball/22 fotball) i oktober/november 2010. Utilstrekkelig 25(OH)D-nivå ble definert som <80 nmol/l. Utøverne registrerte kostholdet sitt med vekt i fire dager. Bruk av kosttilskudd og solvaner ble rapportert ved intervjuer-baserte spørreskjemaer. **Resultater:** Gjennomsnittlig serum 25(OH)D-konsentrasjon var 99 (SD=32) nmol/l. Utilstrekkelig vitamin D-nivå ble funnet hos 27 prosent av utøverne. Håndballspillerne hadde signifikant høyere serum 25(OH)D-nivå enn fotballspillerne (p=0.001). Deltakerne som kun oppholdt seg i Norden om sommeren, hadde signifikant lavere 25(OH)D-nivå enn deltakerne som reiste utenfor Norden (p=0.039). Håndballspillerne reiste signifikant mer utenfor Norden enn fotballspillerne (p=0.004). Vitamin D-inntaket var under anbefalt nivå, 7,5 µg/dag, for 73 prosent av alle deltakerne. **Konklusjon:** I denne studien, gjort på kvinnelige utøvere fra høy nordlig breddegrad, var gjennomsnittlig 25(OH)D-nivå om høsten tilsvarende optimalt nivå, og utilstrekkelig 25(OH)D-nivå ble funnet hos en av fire utøvere. Å reise utenfor Norden om sommeren så ut til å være den faktoren av størst betydning for 25(OH)D-nivåene. Siden deltakerne bor ved høy nordlig breddegrad og siden vitamin D-inntaket var under anbefalt nivå for ¾ av deltakerne, er det sannsynlig at 25(OH)D-nivåene deres vil synke i løpet av vinteren. Derfor er det nødvendig å diskutere om generell screening av 25(OH)D-konsentrasjon for kvinnelige toppidrettsutøvere bør anbefales.

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Abbreviations and definitions

25(OH)D	25-hydroxyvitamin D
$1,25(OH)_2D$	1,25-dihydroxyvitamin D
7-DHC	7-dehydrocholesterol
AI	Adequate Intake, usual intake for an individual at this level or above has a low probability of nutrient inadequacy
Anthropometry	Weight (self-reported), height (self-reported), BMI (calculated)
BMD	Bone Mineral Density
BMI	Body Mass Index (kg/m^2)
BMR	Basal Metabolic Rate
CHO	Carbohydrate
DBP	Vitamin D Binding Protein
DRI	Dietary Reference Intake
f	Female
Female Athlete Triad	The interrelationships among energy availability, menstrual function and BMD that may have clinical manifestations including eating disorders, functional hypothalamic amenorrhea, and osteoporosis.
g	Gram
IOM	Institute of Medicine, US
IU	International Units (40 x µg vitamin D)
KBS	Kostberegningssystemet (Norwegian Food Database)
kg	Kilogram
kJ	kilojoule, measure of energy in the International System of Units
m	Male
µg	Microgram
MJ	MegaJoule, 1000kJ
N	Normally (distributed)

n	Number of subjects
NHANES	National Health and Nutrition Examination Survey, NHANES III; a cross-sectional survey administered to a nationally representative sample of non-institutionalized civilians aged 2 months and older.
NNR	Nordic Nutrition Recommendations
Olympiatoppen	Resource center for Norwegian top-level sports
PAL	Physical activity level
PASW	Predictive Analytics SoftWare
PTH	Parathyroid hormone
RCT	Randomized Controlled Trial
RDA	Recommended Daily Allowance
REK	Regional Committee for Research Ethics (Regional Etisk Kommité)
SD	Standard Deviation
UL	Upper Level
URTI	Upper respiratory tract infection
US	United States (of America)
UVB	Ultraviolet B light
VDR	Vitamin D Receptor
VDRE	Vitamin D responsive elements
Vitamin D_2	Ergocalciferol, derived from UVB exposure precursor ergosterols in fungi and yeast
Vitamin D_3	Cholecalciferol, animal form derived from UVB exposure
Vitamin D winter	The period when there is not sufficient UV radiation outdoors for a person to produce vitamin D
Vmax	The velocity of an enzyme at maximum concentration of substrate

1. Introduction

The interest for the role of vitamin D for health status is increasing, as vitamin D deficiency is now recognized as a pandemic [1]. The two principal driving forces for the increased interest can be traced to 1) the worldwide trend to nutritional vitamin D insufficiency and 2) new knowledge regarding the non-hormonal, intracrine and paracrine actions of circulating vitamin D metabolites [2]. Whether and how much vitamin D influences functionality are debated, not only as a factor for bone health, but also as possible impact on many organ systems. It is of great interest to find the optimal vitamin D levels, measured as serum levels of 25-hydroxyvitamin D (25(OH)D), in different population groups. Most research has been carried out on populations of elderly and a low 25(OH)D level is often found among these [3].

In the field of sports nutrition the necessary 25(OH)D level, of athletes, especially among females, has been debated. There is some evidence that an adequate 25(OH)D level is positively correlated to exercise performance. However, how vitamin D enhance skeletal muscle function, and what the optimal 25(OH)D level (if any) to maximize exercise performance is, still remains as unanswered questions [4].

The Female Athlete Triad refers to the interrelationships among energy availability, menstrual function and bone mineral density (BMD) [5]. High prevalence of menstrual dysfunction has been found for the female athlete, and this increases the risk of stress fracture and injury [6]. Reduced rate of bone formation in athletes with amenorrhea is often caused by estrogen deficiency accompanied by chronic undernutrition [5]. Apparently the bone loss of premenopausal amenorrheic women is not fully reversible, as Nattiv et al [5] states that no pharmacological agent adequately restores bone loss or corrects metabolic abnormalities that impair health and performance in athletes with amenorrhea. Different aspects of the Female Athlete Triad have been evaluated for female football players in Norway [7], but the vitamin D status of athletes with the Triad disorders has not been investigated. This could be of interest due to the impact of vitamin D on calcium metabolism and bone health.

1.1. Vitamin D physiology

Vitamin D is produced by the skin or absorbed through the intestine [8]. The major source is skin production by an ultraviolet B (UVB) -light mediated, photolytic, non-enzymatic reaction that converts 7-dehydrocholesterol (7-DHC) to previtamin D_3 (Figure 3). Subsequent, there is a further non-enzymatic, thermal isomerization conversion to vitamin D_3 [2]. Vitamin D_3 is transported from the skin to general circulation, and is taken up by the liver. In the hepatic parenchyma, vitamin D_3 is converted to 25(OH)D_3, by cytochrome P450s [2].

Dietary vitamin D includes both D_3 (cholecalciferol, animal form) and D_2 (ergocalciferol, derived from UVB exposure precursor ergosterols in fungi and yeast). They are both readily absorbed into intestinal mucosa and transported to the liver where they are converted in the same way as vitamin D_3 from sunlight [9].

Vitamin D acquired through sun exposure or through oral consumption is metabolized within about three days, by the liver to 25(OH)D [10]. The vitamin D metabolite 25(OH)D is a prohormone to the active form 1,25-dihydroxyvitamin D (1,25$(OH)_2$D) [2]. In the kidney, 1α hydroxylase activates 25(OH)D to 1,25$(OH)_2$D, mainly regulated by parathyroid hormone (PTH), hypophosphatemia and low dietary calcium [8]. Production and release of PTH are regulated by calcium-sensing receptors on parathyroid cells. PTH interacts with its receptor on proximal tubular epithelial cells of the kidney and signals an increase in CYP27B1 gene expression and conversion of 25(OH)D to 1,25$(OH)_2$D [2].

The active 1,25$(OH)_2$D binds to a vitamin D receptor (VDR) to carry out its functions. VDRs are ligand-dependent transcription factors which recognize specific DNA sequences known as vitamin D response elements (VDREs) [11]. VDRs are present in more than 30 different tissues, as cytosolic or nuclear and/or membrane-bound receptors [12] indicating multiple functions of the vitamin.

Serum 25(OH)D level is the sum of circulating 25(OH)D_3 and 25(OH)D_2. The serum level of 25(OH)D_3 is much higher than that of 25(OH)D_2 [13], since all vitamin D derived from sunlight and the major part of dietary vitamin D originate from the

vitamin D_3 form (vitamin D_2 is mostly supplemental). See figure 1 for metabolic pathways for vitamin D. In the following 25(OH)D is used for the sum of 25(OH)D_3 and 25(OH)D_2 since they have similar properties.

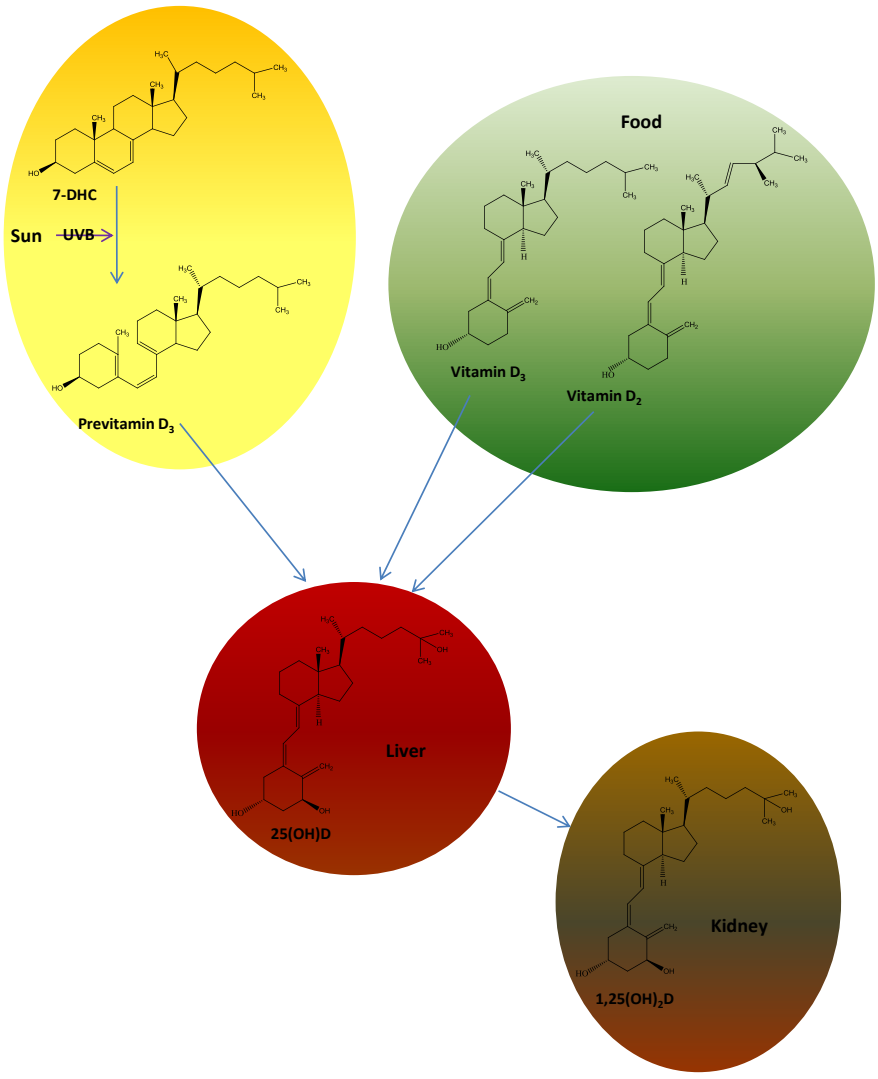

Figure 1: Vitamin D metabolism

Vitamin D_3 from skin production and diet, and some vitamin D_2 from supplements and diet, enters circulation and is taken up by the liver [9]. Serum 25(OH)D produced in the liver includes both 25(OH)D_3 and 25(OH)D_2, however there is substantially higher serum levels of 25(OH)D_3 than 25(OH)D_2 (when supplemental vitamin D_2 is not used) [13]. In the kidneys 25(OH)D is activated into 1,25(OH)$_2$D under regulation by mainly PTH, calcium and phosphorus [8].

25(OH)D is the most often occurring and most stable metabolite of vitamin D in human serum. Vitamin D binding protein (DBP) both binds and transports 25(OH)D and $1,25(OH)_2D$ in serum [3]. DBP is synthesized in the liver and circulates at a concentration that is in excess of normal circulating vitamin D metabolite concentrations. DBP has a higher affinity for 25(OH)D than $1,25(OH)_2D$, binding 99% of 25(OH)D, which is present in circulating concentrations 1000 times those of $1,25(OH)_2D$. The half-life of $1,25(OH)_2D$ is <4 hours, while the 25(OH)D half-life is 2–3 weeks [3]. When sun exposure is restricted, the decline in serum 25(OH)D progresses slowly, with a half-life of at least two months [14].

Even with a marked vitamin D deficiency, elevated PTH levels will maintain the conversion of 25(OH)D to $1,25(OH)_2D$, thereby sustaining $1,25(OH)_2D$ levels within normal ranges, despite low reserves [15]. The production of 25(OH)D is not regulated (as $1,25(OH)_2D$ is by PTH) and 25(OH)D level thus reflects both absorption from the diet and skin synthesis [16]. Serum 25(OH)D concentration is found to be the most valid estimate for determination of vitamin D status in humans [16-18].

1.2. Vitamin D functions that could affect athletes

1.2.1. Skeletal functions

Active vitamin D ($1,25(OH)_2D$) regulates genes in the gut to promote intestinal calcium and phosphorus absorption [2]. Without vitamin D, only 10-15% of dietary calcium and 60% of phosphorus are absorbed, while absorption efficiencies can be increased to 30-40% and 80%, respectively, with adequate vitamin D [19]. Vitamin D activates genes in bone, and liberates calcium and phosphorus from the mineral phase of bone [2]. The National Health and Nutrition Examination Survey (NHANES) III was conducted between 1988 and 1994 to study the health and nutritional status of non-institutionalized residents in the United States (US). Complete data were available for 18 883 participants in NHANES III and 13 369 participants in NHANES 2001-2004 [20]. A positive relationship between BMD and 25(OH)D level was found in the NHANES III study [21].

According to Sundgot-Borgen and Torstveit [7], a number of studies have reported a significant decrease in vertebral BMD among young female athletes with menstrual dysfunction. The vitamin D status may be of interest for the Female Athlete Triad, since insufficient vitamin D status could enhance the negative effect of menstrual dysfunction on bone health, via its effects on calcium metabolism. However, more research is needed to determine if higher intakes of vitamin D and calcium can increase BMD and reduce fractures in female athletes with the Triad disorders [5].

An extra renal production of $1,25(OH)_2D$ also takes place and thus vitamin D may play an important role in cell differentiation, proliferation and immune function in different tissues. In contrast to renal 1α hydroxylase, extra renal 1α hydroxylase does not respond to stimulation by PTH, and vitamin D could play a role in physiologic processes independently of its well-known role in calcium metabolism [3].

1.2.2. Muscle function

Already in 1975 Birge and Haddad [22] showed that exogenous 25(OH)D affected de novo protein synthesis in muscle of rats, concluding that it acts directly on muscle to increase protein synthesis. The findings have been confirmed in studies of human muscle biopsies. One example is a study of 12 vitamin D-deficient patient biopsies, which showed atrophy of Type II muscle fibers before treatment and significant improvement after treatment with supplemental vitamin D [23].

In a review on vitamin D and human skeletal muscle, Hamilton [24] concluded that the identification of VDR in skeletal muscle, its various polymorphisms and variable expression with aging have provided some insight into the complex mechanisms of possible vitamin D skeletal muscle functions. Both nuclear and membrane-bound VDRs are found in skeletal muscle, resulting in both genomic and non-genomic effects of vitamin D with impact on calcium metabolism and protein transcription [24].

Ward et al [25] found a positive relationship between 25(OH)D level and muscle function in post-menarchal adolescent girls. They suggest that the mechanism can be

multifactorial due to genomic (protein synthesis due to nuclear VDRs) and non-genomic (calcium and phosphorus transport due to VDRs on cell membrane) effects of active vitamin D. In a randomized controlled trial (RCT) of vitamin D supplementation, El-Hajj et al [26] observed an increase in whole body lean mass (a surrogate measure of muscle mass) in pre-menarchal girls who received the supplement.

Three cross-sectional community studies [27-29] found direct correlation between 25(OH)D levels and physical performance (Table 1). The studies evaluated physical performance by e.g. walking tests, sit-to-stand tests, muscle strength and fractures. Increase in 25(OH)D levels between 10 and 30 mg/ml (25 and 75 nmol/l) gave the most dramatic differences in performance. They also found a threshold level of 25(OH)D at 40-50 ng/ml (100-125 nmol/l), above which further improvement in neuromuscular performance was not seen. Important to mention is that these studies were performed on population groups of elderly. A case-control study of younger subjects [30] showed that vitamin D supplementation gave great improvement in muscle function, evaluating quadriceps muscle power, in 55 severely vitamin D deficient younger Arab women living in Denmark. If vitamin D really improves muscle function, possible effects on performance by 25(OH)D levels in athletes needs to be verified.

1.2.3. Immune and inflammatory functions

Vitamin D's functions are not restricted to the musculoskeletal system. Recent evidence has also linked low 25(OH)D levels to various non-skeletal, chronic and autoimmune diseases including cardiovascular disease, hypertension, diabetes, inflammatory bowel disease, depression, multiple sclerosis, rheumatoid arthritis and certain types of cancer [12;19]. A meta-analysis by Autier and Gandini [31], reviewing vitamin D supplementation and total mortality investigating 18 RCTs, indicates that vitamin D, even in relatively low doses, reduces total mortality.

Vitamin D might be an important component in immune function and inflammatory modulation [12;32]. Ginde et al [33] recently performed a secondary analysis of the NHANES III study, and examined the association between 25(OH)D level and recent upper respiratory tract infection (URTI). They demonstrated that the prevalence of URTIs increased significantly as the serum 25(OH)D level dropped, and was greatest during winter months when 25(OH)D levels were at their lowest.

Based on our current understanding of vitamin D's role in bone health, muscle, inflammation and immunity (innate and exercise related), it is plausible that suboptimal 25(OH)D levels increase the risk of inflammatory injuries as well as the susceptibility to common upper respiratory tract infection and other illnesses. The studies have been mostly performed on the elderly, but these effects may also apply to other population groups. Thus, it is likely that compromised 25(OH)D levels can affect an athlete's overall health, ability to train and perform [34;35].

1.3. Setting recommendation for serum 25(OH)D levels

Both ng/ml and nmol/l are used as units for 25(OH)D level, and the conversion factor from ng/ml to nmol/l is 2.496. Natural levels, that is, levels found in humans who live or work in the sun, are ≈50-70 ng/ml (≈125-175 nmol/l) – which are levels attained by only a small fraction of modern humans [10].

Serum PTH have been found to rise significantly once serum 25(OH)D level is <30 ng/ml (<75 nmol/l), and a level of 20-30 ng/ml (50-75 nmol/l) is in recommendations considered to represent vitamin D insufficiency, whereas a 25(OH)D level <20 ng/ml (<50 nmol/l) is defined as vitamin D deficiency [1;2]. In 2011 the new report on dietary requirements for calcium and vitamin D from the Institute of Medicine (IOM), US, was published [36]. The Committee concluded, after a careful review of available literature, that serum 25(OH)D levels of 16 ng/ml (40 nmol/l) cover the requirements for approximately half the population, and levels of 20 ng/ml (50 nmol/l) cover the requirements for at least 97.5% of the population and therefore still can be considered sufficient as the lower value of the reference level [36]. However, many authors have

recently discussed whether a much higher 25(OH)D level is needed. Desirable and optimal serum 25(OH)D levels for performance and bone health found in various studies are listed in Table 1.

Table 1: Desirable and optimal serum 25(OH)D levels for performance and bone health

Metabolic measurement/ Health outcome	*Recommendation (nmol/l, desirable/optimal)*	*References*
PTH plateau	70-100	Heaney [37], Dawson-Hughes et al [38], Kuchuk et al [39], Thomas et al [40], Chapuy et al [41], Peacock [42]
Maximum calcium absorption	80-85	Heaney et al [43]
Vmax[a]	>100	Hollis et al [44]
Secondary hyperparathyroidism	>100	Laaksi et al [45]
Decrease in osteoporotic fractures	80	Heaney [46]
Bone mineral density	>75/ 90-100	Bischoff-Ferrari et al [47]
Physical performance in elderly[b]	25-75 (↑performance) / 100-125 (threshold)	Wicherts et al [29], Bischoff-Ferrari et al [27], Gerdhem et al [28]

[a] Vmax; the velocity (of 25-hydroxylase) at maximum concentration of substrate.
[b] Physical performance tests; walking/sit-to-stand/balance tests, muscle strength and fractures

Epidemiological studies finding associations between 25(OH)D levels and different metabolic measurements and health outcomes have indicated a range of 75–100 nmol/l to be favorable. The use of health outcomes as endpoints have not, however, been used in connection with recommendations for 25(OH)D levels [48]. The main metabolic measurements and health outcomes that have been investigated have been the 25(OH)D level needed to optimize calcium absorption, the level leading to maximal suppression of PTH, or the level reducing osteoporotic fractures.

Studies on the 25(OH)D level needed to optimize different metabolic measurements have been carried out by several investigators. Heaney et al [43] found that intestinal absorption of calcium increased when the 25(OH)D level increased from 30 to 80–85 nmol/l (Table 1). Among others (Table 1), Heaney [37] found that PTH levels were at a minimum at 25(OH)D levels of 70-110 nmol/l. Hollis et al [44] concluded that optimal vitamin D status may occur when the Vmax (the velocity at maximum concentration of substrate) of the 25-hydroxylase (the enzyme converting vitamin D_3 to 25(OH)D) appeared to be achieved, which occurred when circulating 25(OH)D exceeded 100 nmol/l (Table 1).

How the 25(OH)D level influence multiple health outcomes has also been investigated. Holick and Chen [1] stated that circulating 25(OH)D >75 nmol/l is required to maximize vitamin D's beneficial health effects. Bischoff-Ferrari et al [47] examined multiple health outcomes, such as BMD (Table 1), and concluded that the most advantageous 25(OH)D levels are above 75 nmol/l, and optimal between 90 and 100 nmol/l. Heaney [46] found that osteoporotic fractures were reduced when 25(OH)D was increased to near 80 nmol/l (Table 1). Laaksi et al [45] concluded that there is strong evidence that, to avoid secondary hyperparathyroidism and the resulting increased calcium release and bone turnover rate, 25(OH)D level should be >100 nmol/l (Table 1).

All the above mentioned studies and other studies in Table 1 suggests a level higher than current recommended 25(OH)D level. Several authors suggest that vitamin D deficiency or insufficiency should be defined as levels <80 nmol/l [34;49-52], while others suggest <75 nmol/l [1;10]. For athletes 80 nmol/l has been used as cut-off for insufficiency in a recent study [34]. As of yet there is no consensus among researchers, and the cut-off for 25(OH)D sufficiency is still set at 50 nmol/l in American and Nordic recommendations.

1.4. Predictors of 25(OH)D level

Predictors of 25(OH)D level are shown in Figure 2.

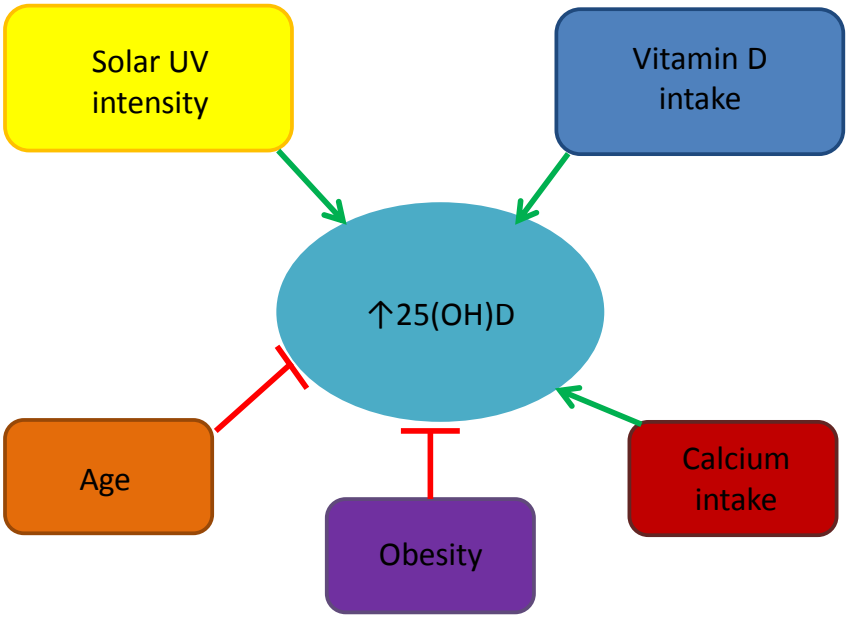

Figure 2: Predictors of 25(OH)D level
Green arrows indicate positive prediction. Red lines indicate negative prediction.

Sun

The major source of vitamin D for most humans is exposure to sunlight [1;32]. Usually, between 50% and 90% of vitamin D in the body results from the production in the skin and the remainder is from the diet [53]. Solar UV intensity and hence skin production of vitamin D is affected by factors such as latitude, season, time of the day, melanin content of the skin, age, clothing and sunscreen use [8;19;32]. Persons with high concentration of melanin in their skin need up to 10 times longer UVB exposure times to generate the same 25(OH)D deposits as fair-skinned [19;32].

At higher latitudes there is a seasonal variation in the 25(OH)D level. Wintertime latitude greater than 37˚ north or south decrease endogenous vitamin D synthesis and

bioavailability due to a marked decrease in the number of UVB photons reaching the earth's surface [54]. Already in 1988, Webb et al [55] found that from November throughout February, solar UVB was insufficient to synthesize vitamin D in Boston (42°N).

The period when there is not sufficient UV radiation outdoors for a person to produce vitamin D is termed the ''vitamin D winter''[56]. Webb and Engelsen [57] estimated required UV exposure times to obtain approximately the equivalent of 10 µg (400 IU). They found for latitude 60°N, as in the present study, that vitamin D cannot be produced from sunlight between mid-October until beginning of March.

Lack of significant amounts of vitamin D in most adult diets, on-going catabolism of body stores and declining UVB radiation in autumn, cause serum 25(OH)D levels to decline as autumn progresses and reaches its lowest value in winter [58]. Hyppönen and Power [59], who studied hypovitaminosis D in British adults (50-60ºN), found serum 25(OH)D levels to peak in September and be at their lowest from January throughout April. Time spent outdoors was strongly correlated with 25(OH)D levels during the summer and autumn in this study, but no correlation was apparent during the winter months, suggesting the importance of sun exposure for serum 25(OH)D levels.

Brustad et al [60] found in a Norwegian study that when excluding subjects that had been either on sun holiday or used sun-bed, the 25(OH)D levels were stable at around 40 nmol/l (16 ng/ml). The main finding in their study was the absence of a clear seasonal variation pattern in the serum levels of 25(OH)D. Other studies from Norway show only a modest difference between summer and winter 25(OH)D levels at around 10 nmol/l [61;62] and less [63]. Brustad et al [60] conclude that the generally high dietary intakes of vitamin D, especially in winter, mask largely the effect of seasonal variation in UV-exposure. This contradicts the claims that solar UV radiation is the major source of vitamin D.

The link between physical performance and 25(OH)D levels has been investigated by several groups. Most of the consistent literature indicates that physical as well as athletic performance have the same pattern of seasonal fluctuation as 25(OH)D; peaking when 25(OH)D levels peak, declining as they decline, and reaching the lowest level when 25(OH)D levels are at their lowest [58]. Such seasonal changes in physical fitness could be due to seasonal changes in total time spent exercising. However, Hettinger and Muller [64] controlled for time spent exercising and found a distinct seasonal variation in the trainability of musculature by studying wrist flexor strength in subjects undergoing daily training. Even though the 25(OH)D level and physical performance have similar seasonal fluctuation pattern, a true relationship between the two factors is yet to be established. Dark-skinned athletes and athletes training mainly indoor, who live at high latitudes, wearing extensive clothing, regularly using sun block, or consciously avoiding the sun, are all at risk for vitamin D deficiency [58].

Obesity

Low 25(OH)D levels have been observed in association with obesity. Obesity-associated vitamin D insufficiency is likely due to the increased body fat to store vitamin D, leading to a decrease in circulating levels [2]. Obesity does not affect the capacity of the skin to produce vitamin D_3, but the release of vitamin D_3 from the skin into the circulation may be altered due to more storage in subcutaneous fat. Obese individuals have >50% decreased bioavailability of vitamin D_3 from skin synthesis compared to non-obese individuals [65].

Age

Increasing age is a known negative predictor of 25(OH)D levels, due to a decrease in epidermal stores of 7-DHC [66]. Skin production of previtamin D_3 has been found to decrease gradually throughout life, but not substantially before becoming elderly, with a 63% decrease in 77 year old compared to 8 year old skin [66]. Another study found that a 70 year-old has ≈25% of the 7-DHC of that of a young adult and thus has a 75%

reduced capacity to synthesize vitamin D_3 in the skin [67]. Need et al [68] also found that 25(OH)D levels fell significantly after the age of 69.

Diet and supplements

In a review of global vitamin D intake, Calvo et al [69] state that there is growing evidence that strong association between 25(OH)D level and the reduced risk of chronic diseases can be linked to vitamin D intake, as well as sun exposure. Vitamin D intake has previously been found to have positive correlation to 25(OH)D levels in subjects with low sun exposure [61;70;71]. Norwegians appear to have higher 25(OH)D levels compared to populations of other Nordic countries as well as countries further south [72]. Jorde and Bønaa [73] found 15 596 Norwegian men and women aged 25-69 to have a daily vitamin D consumption of 6.8 µg and 5.9 µg respectively. Norway has little fortification of foods (only margarine and certain types of milk), but Norwegians have relatively high dietary vitamin D intake due to high fish consumption, daily contributing to an intake of 1.8 µg and 1.5 µg vitamin D for men and women, respectively. Supplements containing vitamin D, especially cod liver oil, constitute a large contributor to the vitamin D intake in Norway (42% for men and 49% for women) [73].

To which extent the amount of dietary vitamin D is sufficient to influence health outcomes such as BMD has been discussed. Meier et al [74] found supplementation with oral vitamin D_3 (12.5 µg/day) and calcium (500 mg/day) during winter to prevent seasonal changes in bone turnover and bone loss in healthy adults living at latitude 49.5°N. Tenforde et al [75] reviewed studies on stress fractures in the young athlete, and evaluated calcium and vitamin D supplementation as possible prevention factors. They concluded that the role of increased vitamin D intake in prevention of stress fractures and improved BMD is currently not evident. They found only one study investigating the effect of vitamin D supplementation on stress fracture in athletes. In an eight week RCT on female navy recruits, Lappe et al [76] found that the group supplementing with 2000 mg calcium and 800 IU (international units) (20 µg) vitamin D had 20% reduced fracture injuries compared to the placebo group.

Dietary calcium intake is thought to affect 25(OH)D levels. A low calcium intake causes an increase of serum PTH and serum 1,25$(OH)_2$D, thereby decreasing the half-life of serum 25(OH)D [77]. Thus, a high calcium intake can increase 25(OH)D levels by prolonging the half-life.

1.5. Recommended intake

The Dietary Reference Intake (DRI) values for vitamin D were initially set by the American IOM in 1997 [78]. Recommended intake was set to an Adequate Intake (AI) value of 5 µg for adults aged 19-50 (Table 2). The AI was based on the level of dietary vitamin D to prevent a wintertime drop [78]. However, two studies, one providing dose–response data [79] and the other linking maintenance of 25(OH)D levels to prevent bone loss [74], suggest that to prevent a wintertime drop, at least 12.5 µg/day are required. To ensure acceptable 25(OH)D level all year around, the Nordic Nutrition Recommendations (NNR) 2004 increased the daily vitamin D intake recommendation for the age group 2-60 years from 5 µg to 7.5 µg [48] (Table 2).

In a study from 2010, Hall et al [80] estimated that a daily intake of 28-64 µg would be needed in winter to maintain 25(OH)D levels of 20-30 ng/ml (50-75 nmol/l) for people of European ancestry with low sun exposure. The new public health report on DRIs for calcium and vitamin D from the American IOM [36], released on November 30th 2010, updates the 1997 IOM report. Recommended Dietary Allowance (RDA) is now 15.0 µg/day (600 IU/day) for persons of 1–70 years, corresponding to a 25(OH)D level of at least 50 nmol/l which meets the needs for at least 97.5% of the population according to the IOM (Table 2). The recommendation is based on available data across ages under conditions of minimal sun exposure. Table 2 presents American and Nordic recommendations for dietary vitamin D intake.

Table 2: Recommended daily dietary vitamin D intake

	Vitamin D (µg/d)	Age group	Reference
IOM 1997	5.0	19-50	[78]
NNR 2004	7.5	2-60	[48]
IOM 2011	15.0	1–70	[36]

The amount of vitamin D required from diet to achieve optimal vitamin D status depends on the initial 25(OH)D level. One µg/day of vitamin D_3 is estimated to give an average increase in serum 25(OH)D of 1.2 nmol/l at low starting serum 25(OH)D levels, but only 0.7 nmol/l or less at the higher starting level of 70 nmol/l [79;81].

Table 3 presents the oral vitamin D_3 intake suggested, by the most experienced researchers in the field, to achieve optimal 25(OH)D levels.

Table 3: Oral vitamin D_3 suggested needed to reach the optimal level of 25(OH)D*

	Optimal 25(OH)D level (nmol/l)	*Oral vitamin D_3 (µg)*
Dawson-Hughes (US)	80	25
Lips (Netherlands)	50	10-15
Holick (US)	75	25
Heaney (US)	80	40
Meunier (France)	75	25
Vieth (Canada)	70	25

* Adopted from table in ref [38] referring to a round table discussion.

In studies from high latitudes, high intakes have been suggested necessary to maintain sufficient 25(OH)D levels in winter. A study on Danish adolescent girls [82] found 18.6 µg to be the vitamin D intake needed for >97.5% of the girls to maintain 25(OH)D levels >50 nmol/l in winter at latitudes >55°N. Even higher intake levels were found necessary for adults in Irish RCTs (51°N and 55°N). The UK Food Standards Agency Workshop Report [83] estimated 28.0 µg/day and 41.1 µg/day to be the intake needed in winter for 97.5% of the population aged 20-40 to maintain 25(OH)D level >50 nmol/l and >80 nmol/l, respectively.

Upper level (UL) for recommended intake set by IOM in 1997 [78] was 50 µg/day. The 2011 report [36] increased the UL to a level of 100 µg/day. Toxicity has not been seen for serum 25(OH)D below 250 nmol/l, a value that would be produced only at continued oral intakes in excess of 250 µg/day [46;84]. High sunlight exposure does not lead to vitamin D intoxication because once previtamin D_3 and vitamin D_3 are made in the skin they can absorb UVB and UVA radiation, resulting in their conversion to several photoproducts with little or no effect on calcium metabolism [85]. The maximum amount of vitamin D production from sunlight results in 25(OH)D levels similar to those resulting from an oral dose of 250 µg. However, vitamin D production from sunlight is unlikely to exceed ≈125 µg/day in North America and Europe [84].

1.6. Serum 25(OH) levels in the general population and in athletes

Insufficient levels of 25(OH)D are prevalent and increasing all over the world. From 1994 to 2004 the number of persons in America with 25(OH)D levels <30 ng/ml (<75 nmol/l) nearly doubled [2]. The NHANES data from 2005-2006 show that 41.6% of the total and 30.9% of the Caucasian adult population in the US had 25(OH)D levels <50 nmol/l [86]. Table 4 presents 25(OH)D levels at different latitudes for the general population and athletes.

Table 4: Variation of serum 25(OH) levels with season and latitude

Location	*Latitude*	*Population (m=men, f=female), age*	*Level in summer/fall (nmol/)l*[e]	*Level in winter/spring (nmol/)l*[e]	*Reference*
General population[a]					
US, Boston	42°N	m&f, 18-29 yrs, n=69 (subgroup)	90 (25)	70 (25)	Tangpricha et al [87]
Canada, Toronto	43ºN	white f, 18-35yrs, n=322/380 (summer/winter)	76 (28)	58 (24)	Vieth et al [88]
Canada, Calgary	51ºN	m&f, 27-89yrs, n=180	71.6 (23.6)	57.3 (21.3)	Rucker et al [89]
United Kingdom	50-60ºN	white m&f, 45yrs, n=7437	60.3 (59.5, 61.0[f])	41.1 (40.4, 41.8[f])	Hypponen and Power [59]
Norway	65-71ºN	f, 44-59yrs, n=443	61.9 (21.5)	49.5 (15.6)	Brustad et al [61]
Norway, Andenes	69.2ºN	m&f, 20-60yrs, n=60	47	42	Brustad et al [60]
Athletes[b]					
Israel	31.8°N	m&f, 10-30yrs[c], n=98, outdoor/indoor	Whole year: 63.1 (20.7) (f: 58.2 (15.0))		Constantini et al [90]
Australia, Canberra	35.3ºS	f, 10-17yrs, n=18	nd[d]	56 (29-84[g])	Lovell [91]
US, Wyoming	41.3ºN	f&m, college athletes ≥18yrs, n=41, outdoor/indoor	122.3 (41.4)	76.1 (23.5)	Halliday et al [34]
France, Montpellier	43ºN	cyclists, m, 20-39yrs, n=7	83.4 (16.0)	nd[d]	Maimoun et al [92]
Finland, Turku	61ºN	f, 9-15yrs, n=131 athletes + 60 controls	62.9±15.0	33.9±13.9	Lethonen-Veromaa et al [93]

[a] Only studies of general population groups including subjects younger than 50 years are included, since only young athletes are included in our study.
[b] Some studies include both summer and winter values, while some have investigated values only in summer or winter.
[c] Only includes subjects not using vitamin D supplements.
[d] Not determined.
[e] Levels are presented as mean (SD) if not other stated.
[f] 95% confidence interval.
[g] Range.

Although very little is known about the 25(OH)D level in athletes, a few studies suggest that a low 25(OH)D level is also a problem in populations comprising athletes. Willis et al [35] state that a surprisingly high percentage of athletes, especially indoor athletes are probably vitamin D deficient. In a German study [94], 77% of young gymnasts had 25(OH)D levels below 35ng/ml (87 nmol/l), and 37% had levels below 10 ng/ml (25 nmol/l).

Investigating female elite gymnasts in Australia, Lovell [91] (Table 4) found that 15 out of 18 women had levels below 30 ng/ml (75 nmol/l) and six had levels below 20 ng/ml (50 nmol/l). A study of young Finnish female athletes (gymnasts and runners) and non-athletes [93] (Table 4) showed no difference, neither in vitamin D intake nor in serum 25(OH)D levels between athletes and non-athletes. Sixty-seven percent of the subjects had levels <15 ng/ml (<37.5 nmol/l) during winter.

More recently, seven French cyclists (Table 4) training 16 hours weekly were found to have mean 25(OH)D levels of 32 ng/ml (80 nmol/l), which are surprisingly low for athletics in a sport for which sun exposure is common [92]. As much as 91% of young Middle-eastern sportsmen were found to have 25(OH)D levels <50 nmol/l [95]. The study was performed at latitude 25ºN in summer months with sun exposure expected to be high. However, the authors of this study report that because of the day time heat and social factors, the majority of the outdoor training was completed after sunset.

More studies are needed to identify the vitamin D status of elite athletes. In the few studies investigating vitamin D status in athletes, most researchers have found 25(OH)D levels in the lower range. Hence, it is important to increase knowledge on the vitamin D status in the athletic population, in order to be able to discuss the need for nutritional strategies in this field.

2. Aim of this study

Bartoszewska et al [4] ask the questions; "If vitamin D does improve muscle function, what implications does this have on the performance of athletes who have vitamin D deficiency? Is routine screening necessary? What is the role of vitamin D supplementation to enhance performance in these athletes?"

Till now only a few unpublished studies on the 25(OH)D levels of athletes in Norway have been performed. The Norwegian Olympic Sports Center in Oslo, Olympiatoppen, is the resource center for Norwegian top-level sports. The Department of Sports Nutrition at Olympiatoppen now wants to address the possible need for routine screening of 25(OH)D levels in elite athletes in Norway in order to optimize 25(OH)D levels for health and performance.

The aim of the present study was to measure serum 25(OH)D concentrations in female handball and football elite athletes in the south eastern parts of Norway, at latitude 60ºN. We also wanted to address factors associated with 25(OH)D levels in this group, by investigating dietary factors, supplement use and sun exposure. The following questions were raised;

What is the serum 25(OH)D level in female elite athletes in Norway?

Are there differences in the serum 25(OH)D levels between handball and football players?

What is the association between dietary intake, supplement use and sun exposure and serum 25(OH)D level in female elite athletes in Norway?

3. Methods

3.1. Subjects and study design

Data collection was carried out from 20th September until 12th November 2010. The study design is described in Figure 3. After recruitment the subjects came to an individual or group session where instructions of how to perform a four-day weighed diet record and training record were given.

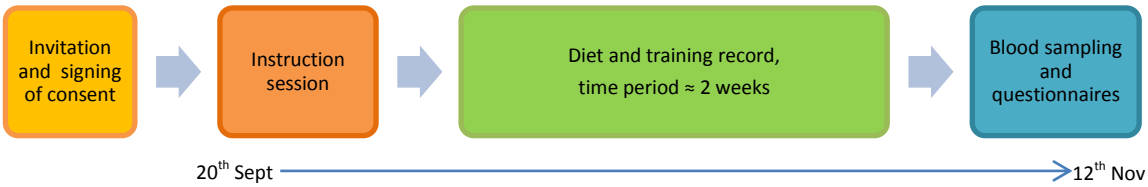

Figure 3: Study design

After finishing the records, the athletes came to Olympiatoppen in Oslo where a blood sample was taken. At the same time the participants orally answered interviewer-administered questionnaires about personal characteristics, supplement use, and sun exposure and behavior. When all results were processed, the athletes received a written evaluation of their diet by mail. Appendix 1 shows an example of a written feedback given to one of the subjects. The subjects were invited to contact the investigator if they had any questions to the feedback or wanted to make an appointment to go through the results.

The three best handball and the three best football teams in the Oslo area were selected for the study. All the football teams and two of the handball teams were from the top league, and the third handball team was highly positioned in the 1st division.

Three inclusion criteria where required to participate in the study. The athletes had to 1) be 18 years or older, 2) play football or handball in a top club in the Oslo area and 3) be free of sickness and injuries in case that would affect the diet in the data collection period.

After approval from the trainers of the respective teams, all players fulfilling the inclusion criteria were invited to participate in the study. Invitation letters (Appendix 2) were sent by mail and/or given in person to a total of 106 athletes. Fifty-seven subjects responded positively to the invitation, participation rate being 54%.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Regional Committee for Research Ethics (REK) (Appendix 3). Written informed consent was obtained from all subjects after they had been fully informed about the study procedures and possible risks.

Fourty-eight subjects decided to complete the study. The nine drop-outs, seven handball players and two football players, all dropped out prior to data collection. All 48 athletes completed the diet- and training records, gave blood samples and answered the interviewer-administered questionnaires. Of the 48 subjects, 26 were handball players and 22 were football players.

3.2. Serum 25(OH)D determination

Blood samples for determination of vitamin D (25(OH)D) were collected between October 5th and November 12th 2010, simultaneously with diet and training record, and questionnaires.

Samples were sent to Fürst Medisinsk Laboratorium (Oslo, Norway) for analysis. Serum 25(OH)D level was measured, with a calibration curve from 25 to 200 nmol/l. (The method was validated as linear from 10 to 500 nmol/l.)

The used liquid chromatography – tandem mass spectrometry (LC-MS/MS) method separately determines 25(OH)D_3 and 25(OH)D_2, and can detect potential artificial intakes of vitamin D_2 (which only contributes in significant amounts from supplements). The 25(OH)D concentrations presented in this study constitute the sum

of 25(OH)D_3 and 25(OH)D_2. The percent relative standard deviation (SD) of the method was ±20% and ±30% for 25(OH)D_3 and 25(OH)D_2, respectively.

The subjects were classified as having insufficient or sufficient levels. The cut-off value used was 80 nmol/l, as also used in a similar study by Halliday et al [34]. The choice of cut-off value is further elaborated upon in discussion of methods (p.63). We used the following categories: deficiency <50 nmol/l, insufficiency ≥50-80 nmol/l, sufficiency ≥ 80-100 nmol/l, and optimal levels ≥100 nmol/l. These categories are based on combination of values used by Grant and Holick [50] and Halliday [34].

3.3. Diet records (Appendix 4)

The instruction sessions were held from September 20th until October 18th. The subjects then received a notebook for diet record and a household scale (Soehnle Electronic Glass Kitchen Scale, which an accuracy of one gram). They were given both practical and written instructions on how to weigh and describe in detail the consumption of food, drinks and supplements, and to measure and note all foods wasted and not eaten. They were especially informed that the purpose of the study was to measure the habitual food intake and that any temptations to change the diet in order to simplify or make the diet healthier should be counteracted.

The subjects recorded their intake of food, drinks and dietary supplements during four days, comprising three weekdays and one Saturday. They recorded all food and drinks, including supplements, and additionally what they ate or drank during training. See Appendix 4 for a template of the record notebook. The setup is based on previous diet records from the Department of Nutrition at the University of Oslo.

Intake of nutrients, with focus on amounts and sources of vitamin D, calcium and energy giving nutrients, was assessed using the Norwegian food calculation system "Kostberegningssystemet" (KBS), version 6 database AE-10. The KBS is based on the official food table "Matvaretabellen" in Norway with addition of other foods, and KBS AE-10 includes new and updated foods from year 2010.

The subjects' energy and nutrient intake both with and without supplements were calculated. Energy and nutrients are presented as average daily intake. Energy giving nutrients are calculated as percent of total daily energy intake, while micronutrients are calculated as daily amounts. To further evaluate the intakes of vitamin D and calcium, the subjects were grouped into above and below recommended intake of both total vitamin D and total calcium. Cut-off value for vitamin D intake was set at 7.5 µg, which is recommended for the Norwegian population aged 2-60, and at 800 mg for calcium intake, which is the dietary recommendation for Norwegian women aged 20-60 [96]. The subjects were also grouped into those with vitamin D and calcium intakes above or below the lower limit, as recommended used in evaluation of results from dietary surveys [96]. Lower limit for vitamin D intake is 2.5 µg/day, and for calcium intake is 400 mg/day.

When handing in the diet- and training record notebook the subjects answered a control questionnaire (Appendix 5). The subjects reported any change in bodyweight, sickness, injuries and medicine use. The scheme has previously been used in studies at Olympiatoppen [97;98].

3.4. Training records (Appendix 4)

A description of the subject's training load was included as a part of the record notebook. Practical and written information on how to fill in the training record was given at the instruction session. See Appendix 4 for a template for the training record in the record notebook. The subjects filled in number of sessions per day and duration, whether the training was performed indoor or outdoor, and food and drinks consumed during training.

3.5. Questionnaires

All questionnaires were collected between October 5th and November 12th 2010.

3.5.1. *Subject characteristics (Appendix 6)*

The subjects were asked for their age, current weight, any change in weight during the diet record period, height, country of birth, parents' country of birth and use of regular medication in an interviewer-administered questionnaire.

3.5.2. *Supplement use (Appendix 7)*

The subject's long term supplement use was assessed by an interviewer-administered questionnaire. The questionnaire is based on questionnaires used in studies at Olympiatoppen [97;98]. The athletes were asked about type of supplements, dosage and frequency at the time of data collection. They also reported whether they used the supplements regularly, the reason for using them and if they had used the supplements since childhood or had just recently started. If the subject did not remember the product name, this information was received by e-mail or phone afterwards.

3.5.3. *Sun behavior (Appendix 8)*

An interviewer-administered questionnaire of sunlight exposure was used. This questionnaire was based on that used in the Norwegian Women and Cancer Study (NOWAC) of female melanoma patients in northern Norway (65-71ºN) by Brustad et al [61]. The variable residing in northern Norway during the summer was in our study adjusted to residing only in Nordic countries in summer.

The participants were asked to recall the number of hours they had spent in daylight last week (the week prior to collection of the blood sample), and average daily number of leisure time hours in daylight in summer. Summer was defined as from the beginning of June till the end of August. The subjects were asked if they had resided in

the Nordic countries during the whole summer 2010, if they had been on a sun holiday abroad since September 2010, and if they have used a sun tanning bed during the past month. The athletes were also asked to report factors for skin type, including hair color and eye color [57], and the use of sun-protection products. Additionally to the questions based on the questionnaire from the NOWAC study, the athletes reported average daily training hours outdoor and indoor in summer.

3.6. Statistical methods

Sample size calculation was based on finding a minimum clinical relevant difference in 25(OH)D level of one SD and a significance level of 0.05 with 90 percent power [99]. Forty participants are required to detect one SD difference between two independent groups, such as grouping the subjects into handball and football players with 20 subjects in each group.

Statistical analyses were performed using Predictive Analytics SoftWare (PASW) for Windows release 18.0.3. All statistical tests used were two-sided and results were considered to be statistically significant at $p < 0.05$.

The variables are presented as means with SDs. Medians are also used in tables containing not normally (N) distributed variables. For N-distributed variables Pearson correlation tests and independent sample t-tests were used. For not N-distributed variables Spearman correlation tests and Mann-Whitney U tests were used. Categorical variables are described as frequencies and proportions. Proportions were compared using Chi-square or Fisher's exact test whichever was applicable.

All variables were statistically analyzed. Associations to 25(OH)D levels and exposure factors were investigated. Multiple regression, assessing the 25(OH)D level, following the enter method, was initially performed. The number of subjects was, however, insufficient to investigate the number of dependent variables of interest. Hence the results from the multiple regression analysis could not be utilized.

4. Results

4.1. *Description of the study population*

Age, anthropometry and hours of training during the record period are shown in Table 5 for the athletes in total and for the handball and football players.

Table 5: Description of the study population

	All (n=48)		**Handball (n=26)**		**Football (n=22)**		
	Mean (SD)	*Median*	*Mean (SD)*	*Median*	*Mean (SD)*	*Median*	*p-value*[a]
Age (yrs)	22.8 (3.9)	21.0	21.5 (2.5)	21.0	24.3 (4.7)	23.5	0.029*[b]
Height (cm)	169.6 (6.3)	169.0	172.4 (6.1)	172.8	166.3 (4.8)	167.3	<0.001*[c]
Weight (kg)	66.4 (6.9)	67.8	69.9 (5.0)	70.0	62.2 (6.5)	60.8	<0.001*[c]
BMI (kg/m^2)	23.0 (1.6)	23.0	23.5 (1.3)	23.4	22.5 (1.7)	22.1	0.017*[c]
Training (hrs/d)	1.5 (0.52)	1.5	1.6 (0.40)	1.6	1.4 (0.63)	1.3	0.159[c]

[a]Difference between handball and football players.
[b] Mann-Whitney U test.
[c] t-tests.
*Significant.

The handball players were significantly younger, taller and had higher weight and BMI than the football players.

Characteristics related to sun exposure for the study population in total are shown in Table 6.

Table 6: Characteristics related to sun exposure of the study population (n=48)

		Number of subjects (%)
Country of birth	*Norway*	43 (90)
	Denmark	2 (4)
	Scotland	1 (2)
	England	1 (2)
	Australia	1 (2)
Acute response to sun without sunscreen	*Always burned*	3 (6)
	Often burned	12 (25)
	Sometimes burned	22 (46)
	Seldom burned	11 (23)
Over time response to sun without sunscreen	*Never tanned*	0 (0)
	Tans with difficulty	3 (6)
	Tans gradually	26 (54)
	Tans easily	19 (40)
Sunscreen use	*Never*	1 (2)
	Sometimes	6 (12)
	Often	26 (54)
	Always	15 (31)
Hair color	*Light blonde*	7 (15)
	Blonde	9 (19)
	Dark blonde	14 (29)
	Brown	18 (38)
Eye color	*Blue*	27 (56)
	Green	10 (21)
	Brown	11 (23)

Most of the subjects were born in Norway (90%). No trend for any of the characteristics related to sun exposure was found.

4.2 Serum 25(OH)D levels

The serum 25(OH)D levels for the athletes in total and for the handball and football players are presented in Table 7.

Table 7: Serum 25(OH)D level

	Mean (SD)			*p-value*[a]
	All (n=48)	**Handball (n=26)**	**Football (n=22)**	
25(OH)D (nmol/l)	99 (32)	112 (26)	84 (18)	0.001*

[a] Difference between handball and football players, t-test.
*Significant

Table 7 shows that the mean 25(OH)D level for the group was 99 (32) nmol/l. Values ranged between 39 and 190 nmol/l (data not shown). The handball players had significantly higher 25(OH)D levels than the football players.

Number of athletes categorized according to 25(OH)D sufficiency levels are presented in Figure 4.

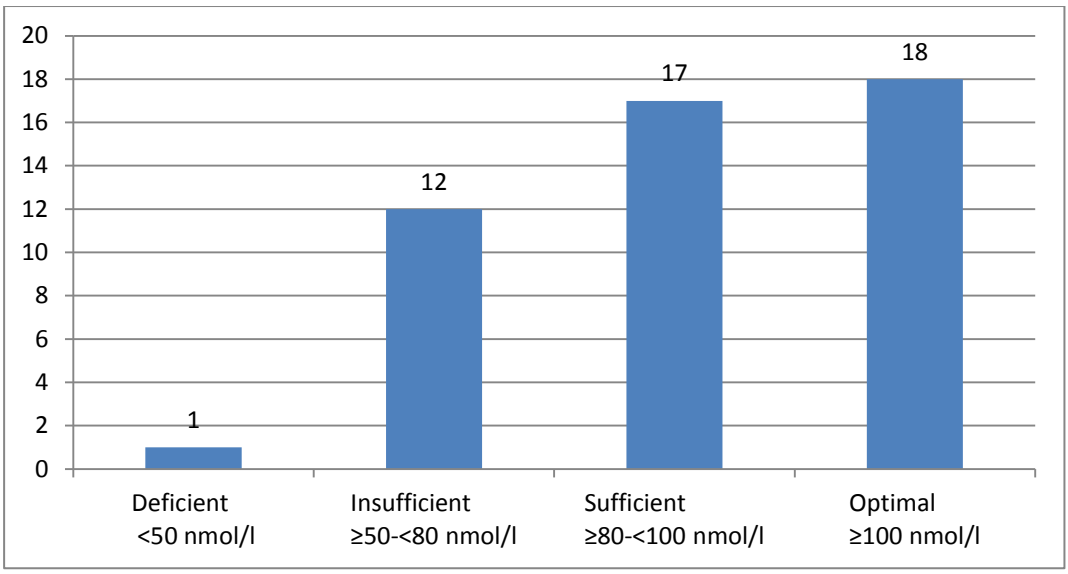

Figure 4: Number of subjects in 25(OH)D level categories, (n=48)

Figure 4 shows that one subject was deficient, 12 (25%) were insufficient, 17 (35%) were sufficient and 18 (38%) had optimal levels. Thus, 13 subjects (27%) had insufficient 25(OH)D levels and 35 subjects (73%) had sufficient levels.

By categorizing in sufficient and insufficient vitamin D status, the significant difference in 25(OH)D levels (Table 7) between the handball and football players was no longer present (p=0.18, chi square test, data not shown).

4.3 Exposure factors

4.3.1 Dietary intake and supplement use

The daily intake of energy and nutrients during the four record days are presented in Table 8 for the athletes in total and for the handball and football players.

Table 8: Daily intake of energy and nutrients

	All (n=48)		Handball (n=26)		Football (n=22)		
	Mean (SD)	*Median*	*Mean (SD)*	*Median*	*Mean (SD)*	*Median*	*p-value*[a]
Energy (MJ/d)	10.0 (2.2)	9.8	10.0 (2.3)	10.1	9.9 (2.2)	9.6	0.813[b]
Protein (%)	16.9 (2.8)	16.4	16.8 (3.0)	16.4	16.9 (2.6)	16.7	0.900[b]
Fat (%)	30.2 (5.1)	30.3	29.7 (5.6)	29.8	30.9 (4.5)	30.6	0.448[b]
CHO (%)	49.6 (5.9)	49.5	50.4 (6.3)	50.2	48.5 (5.3)	48.8	0.264[b]
Sugar (%)	9.2 (4.6)	8.4	9.2 (5.0)	8.2	9.3 (4.1)	8.9	0.892[b]
Alcohol (%)	1.2 (2.0)	0.0	0.85 (1.7)	0.0	1.6 (2.3)	0.0	0.239[c]
Vitamin D excl suppl (µg/d)	3.9 (2.3)	3.4	3.5 (2.1)	3.2	4.4 (2.6)	4.2	0.206[b]
Vitamin D incl suppl (µg/d)	6.1 (5.3)	4.5	6.2 (6.1)	4.4	5.9 (4.4)	5.3	0.605[c]
Calcium excl suppl (mg/d)	970 (38)	900	970 (41)	910	960 (36)	900	0.930[b]
Calcium incl suppl (mg/d)	990 (37)	950	990 (39)	940	990 (36)	950	0.977[b]

[a] Difference between handball and football players.
[b] t-tests.
[c] Mann-Whitney U tests.
*Significant.

Table 8 shows that the median daily vitamin D intake including supplements was 4.5µg. Excluding supplements mean daily intake of vitamin D was 3.9 (2.3) µg.

No difference in energy intake nor in energy giving nutrients between the handball and the football players was found. Also, no difference in vitamin D intake nor in calcium intake between the handball and football players was found.

Number of athletes categorized according to recommended vitamin D and calcium intakes are presented in Figure 5.

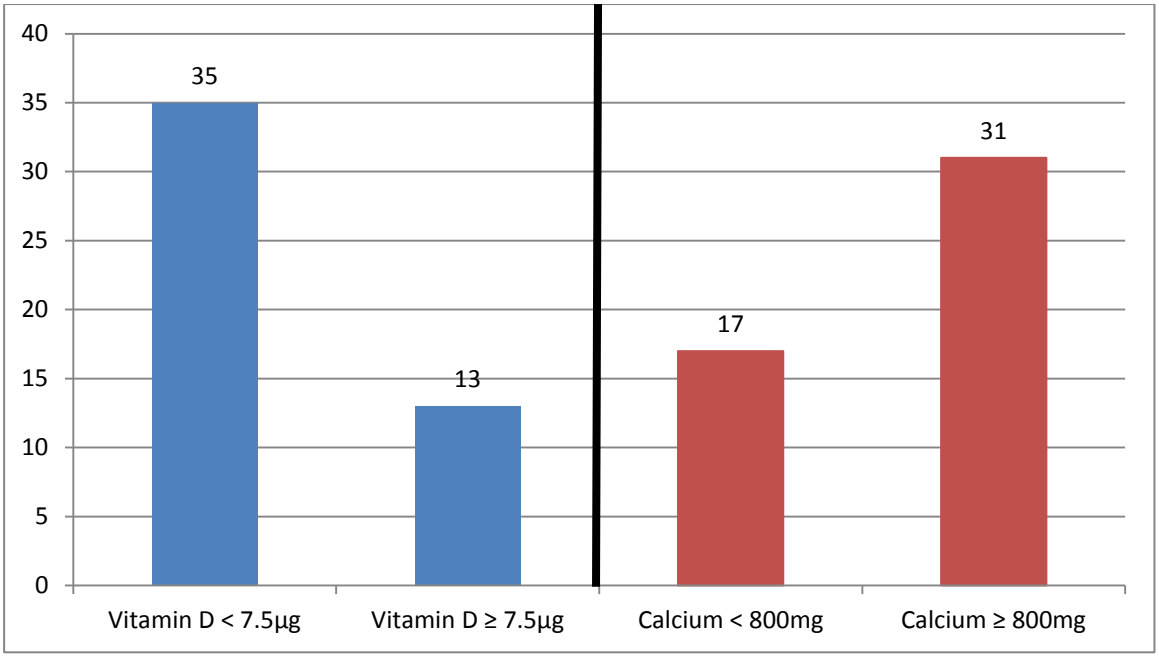

Figure 5: Number of subjects above or below recommended total vitamin D and calcium intake, (n=48 for both vitamin D and calcium intakes)

Of the subjects in total, 13 (27%) had a total daily vitamin D intake ≥7.5 µg, while 35 (73%) had a total vitamin D intake <7.5 µg. Ten (21%) of the subjects had a vitamin D intake below the lower limit of 2.5 µg/day (data not shown).

Thirty-one (65%) of the subjects had a total daily calcium intake ≥800 mg, while 17 (35%) had a total calcium intake <800 mg (Figure 5). Two (4%) of the subjects had a calcium intake below the lower limit of 400 mg/day (data not shown).

There were still no differences in vitamin D intake or calcium intake between the handball and football players when grouping in above or below recommended intake of the respective nutrients (data not shown).

Food sources of vitamin D

For the mean vitamin D intake, excluding supplements, for the athletes in total, the largest source was found to be fish with a mean of 1.4µg (36%) vitamin D per day, whereof 0.69µg (18%) was fatty fish. The second largest source was egg, with a mean contribution of 0.85µg (22%) vitamin D per day. The third largest source was butter and margarine contributing to a mean of 0.70µg (18%) vitamin D per day. These three food sources contributed to 76% of the dietary intake of vitamin D excluding supplements (data not shown).

Vitamin D supplements

In the interviewer-administered questionnaire (Appendix 7) 23 (48%) subjects reported of regular supplement use. Twelve (25%) of the subjects reported using a vitamin D supplement on a regular basis, and among these 10 (21%) subjects reported using cod liver oil. Most of the subjects reported that they used vitamin D supplements regularly the whole year, and that they did not supplement since childhood but started supplementing within the last years (data not shown).

During the four diet record days, 11 of the athletes used vitamin D supplements. Vitamin D intake for the athletes using and not using vitamin D supplements during the record days are presented in Table 9.

Table 9: Vitamin D intake for subjects using and not using vitamin D supplements during the diet record

	Subjects using a vitamin D supplement (n=11)	**Subjects not using a vitamin D supplement (n=37)**	*p-value*[a]
Vitamin D intake (mg/d) – *mean (SD)*	13.5 (5.6)	3.8 (2.4)	<0.001*

[a] To assess the difference between the subjects using a vitamin D supplement and those not, independent sample t-test was used.
*Significant.

The vitamin D intake (including supplements) was significantly higher for the subjects using vitamin D supplements during the diet record days than the subjects who did not. No difference was found in use of vitamin D supplements between the handball and the football players (data not shown).

Mean daily vitamin D intake from supplements among the subjects using a vitamin D supplement during the record period was 9.4 (5.8) µg with a range from 2.5µg to 23.8µg (data not shown). The highest intake was an outlier, and the second highest daily intake amount of vitamin D from supplements was 12.5µg.

4.3.2 Sun behavior

Sun exposure and behavior factors that might influence serum 25(OH)D levels are presented in Table 10.

Table 10: Sun exposure and behavior factors

	All (n=48)		Handball (n=26)		Football (n=22)		
	Mean (SD)	*Median*	*Mean (SD)*	*Median*	*Mean (SD)*	*Median*	*p-value*[a]
Sun last 7 days (hrs/d)	1.9 (1.3)	1.9	1.3 (1.0)	1.0	2.7 (1.2)	2.4	<0.001*[b]
Sun in summer (hrs/d)	4.6 (2.0)	4.8	4.7 (2.0)	5.0	4.4 (2.0)	4.0	0.572[c]
Training outdoor in summer (hrs/d)	1.4 (0.70)	1.1	1.0 (0.38)	1.0	1.9 (0.71)	1.8	<0.001*[b]
Training indoor in summer (hrs/d)	0.60 (0.52)	0.50	0.91 (0.48)	0.75	0.24 (0.28)	0.18	<0.001*[b]
	No (%)	*Yes (%)*	*No (%)*	*Yes (%)*	*No (%)*	*Yes (%)*	*p-value*[a]
Residing only in Nordic countries in summer	30 (63)	18 (38)	21 (81)	5 (19)	9 (41)	13 (59)	0.004*[d]
Tanning bed use	35 (73)	13 (27)	19 (73)	7 (27)	16 (73)	6 (27)	0.978[d]

[a] Difference between handball and football players.
[b] Mann-Whitney U tests.
[c] t-tests.
[d] chi-square tests.
*Significant.

The football players spent significantly more time outdoor the week prior to blood sampling and trained significantly more outdoor in summer than the handball players. The handball players trained significantly more indoor in summer than the football players. Significantly more of the football players than the handball players resided only in Nordic countries in summer. In other words, going outside of Nordic countries in summer was more prevalent among the handball players.

4.4 Associations between 25(OH)D level and exposure factors

Possible correlations between 25(OH)D level and age, BMI, vitamin D intake, calcium intake and sun exposure factors are presented in Table 11.

Table 11: Possible correlations between serum 25(OH)D levels and characteristics and exposure factors

	All (n=48)		**Handball (n=26)**		**Football (n=22)**	
	Correlation coefficient	*p-value*[a]	*Correlation coefficient*	*p-value*[b]	*Correlation coefficient*	*p-value*[c]
Age (yrs)	-0.081	0.585[d]	0.137	0.504[d]	-0.112	0.619[e]
BMI (kg/m^2)	0.146	0.322[e]	0.144	0.481[e]	-0.267	0.229[e]
Vitamin D incl suppl (µg/d)	-0.114	0.440[d]	0.023	0.910[d]	-0.293	0.186[e]
Calcium incl suppl (mg/d)	0.253	0.083[e]	0.312	0.121[e]	0.244	0.274[e]
Sun last 7 days[f] (hrs/d)	-0.108	0.466[e]	0.409	0.038*[d]	0.008	0.973[e]
Sun in summer[g] (hrs/d)	0.094	0.527[e]	0.131	0.525[e]	-0.083	0.712[e]
Training outdoor in summer (hrs/d)	-0.218	0.137[d]	0.212	0.299[d]	-0.110	0.625[d]
Training indoor in summer (hrs/d)	0.489	<0.001*[d]	0.415	0.035*[d]	0.148	0.510[d]

[a]Correlation to 25(OH)D level for all subjects.
[b]Correlation to 25(OH)D level for handball players.
[c]Correlation to 25(OH)D level for football players.
[d]Spearman correlation coefficient.
[e]Pearson correlation coefficient.
*Significant.

Vitamin D intake (both excluding and including supplements) was not correlated to measured 25(OH)D levels. No difference in total vitamin D intake between those with insufficient and sufficient 25(OH)D level was found (p=0.114, t-test, data not shown). Also no difference in 25(OH)D level between the subjects with total vitamin D above or below recommended intake was found (p=0.981, t-test, data not shown).

Calcium intake (both excluding and including supplements), was not correlated to measured 25(OH)D levels. No difference in total calcium intake between the subjects with sufficient and insufficient 25(OH)D level was found (p=0.12, t-test, data not shown). However, a significant difference in 25(OH)D levels between the subjects with total calcium above and below recommended intake was found (p=0.027, t-test, data not shown). Those with calcium intake ≥800 mg/day had a mean 25(OH)D level of 106 (35) nmol/l, and those with calcium intake <800 mg/day had a mean 25(OH)D level of 87 (23) nmol/l, hence the association was positive.

Table 11 shows that hours in daylight the week prior to blood sampling was significantly correlated to 25(OH)D levels for the handball players, but not for the football players nor for the athletes in total. Training indoor in summer had a significant positive correlation with 25(OH)D levels for the athletes in total. This was also the case for the handball players, while there was no correlation between 25(OH)D level and training indoor for the football players.

Differences in 25(OH)D levels between the athletes grouped in residing only in Nordic countries in summer and not, and the athletes grouped in using and not using tanning bed the last month are presented in Table 12.

Table 12: Serum 25(OH)D levels in groups with different sun exposure factors (n=48)

		25(OH)D (nmol/l) *-mean (SD)*	*p-value*[a]
Residing only in Nordic countries in summer	*No (n=30)*	107 (35)	0.039*
	Yes (n=18)	87 (23)	
Tanning bed use	*No (n=35)*	96 (32)	0.172
	Yes (n=13)	110 (33)	

[a] To assess the difference between those residing only and not residing only in Nordic countries, and the difference between the subjects using and not using a tanning bed, independent sample t-tests were used.
*Significant.

The serum 25(OH)D levels were significantly lower for the subjects residing only in Nordic countries in summer than for those who did not. In other words, those going outside of Nordic countries in summer had significantly higher 25(OH)D levels.

There was no significant difference in the 25(OH)D level between the subjects using and not using tanning bed the month prior to blood sampling.

We wanted to explore whether the lower 25(OH)D levels found for the subjects residing only in Nordic countries in summer was influenced by other exposure factors or not. We grouped the subjects according to residence in Nordic countries only or not, and investigated how possible exposure factors (sub-analysis of variables from Table 11) were associated to 25(OH)D levels within these groups. No exposure factor was found to influence the difference in 25(OH)D level between the subjects residing only in Nordic countries in summer and not (data not shown).

Possible differences in dietary vitamin D intake, dietary calcium intake, supplement use and sun exposure between the subjects residing only in Nordic countries and not were also examined. Table 13 shows the vitamin D intake for the athletes residing only in Nordic countries in summer and not.

Table 13: Vitamin D intake in subjects residing only in Nordic countries in summer and not (n=48)

	Nordic NO (n=30)		**Nordic YES (n=18)**		
	Mean (SD)	*Median*	*Mean (SD)*	*Median*	*p-value*[a]
Vitamin D intake incl supplements (µg/d)	5.5 (5.6)	3.9	7.0 (4.8)	5.8	0.142

[a] Mann-Whitney U test, significance level: p <0.05

For the study sample in total the subjects residing only in Nordic countries in summer apparently had a slightly higher median vitamin D intake including supplements, however the difference was not significant. Neither dietary calcium intake, nor supplement use nor sun exposure were different between the subjects residing only in Nordic countries in summer and those not.

5. Discussion

To the best of my knowledge there are only a few unpublished studies on vitamin D status in athletes in Norway. The results from the present study constitute therefore an important addition to the scarce information on vitamin D status in female elite athletes in Norway.

5.1. *Sample*

5.1.1. *Participation rate*

The participation rate in the present study was 54%. This is quite similar to the 58% reported both by Helle [97] in a study of top-level endurance athletes in Norway and by Burke et al [100] in a study of male and female Australian Olympic team athletes.

There are several explanations for the moderate participation rate in our study. Some of the athletes had previously done diet records with the national team, and they may not have found the reward of getting dietary feedback so useful. The hectic life of an elite athlete with a lot of travelling may also have been a general cause for not participating. The days of recording had to be done in their home area on non-travelling days. Even though the record days did not have to be consecutive, it could be challenging for some of the athletes with matches both with their club and the national team.

To facilitate the recruitment, participants were promised a written evaluation of their diet and the results for their serum 25(OH)D level. The use of a reward has been shown to enhance the participation rate in dietary surveys [101]. Also, the fact that this study was conducted in the regime of Olympiatoppen, may have contributed to a positive attitude towards participation. (Olympiatoppen is the Olympic Sports Center in Norway.)

Of the 57 subjects that initially were included, 48 completed the study, giving a rate of 84%. The tight follow-up during the whole project period, with instructions, and regular mails and phone calls, was probably an important reason for the high rate of subjects completing the study. The nine subjects lost to follow up (drop-outs), all

reported insufficient time as the reason for not continuing the study. Of these subjects seven were handball and two were football players, leading to a more even number of handball and football players in the study population. They all dropped out prior to the data collection, hence it remains unknown if the drop-outs had deviating characteristics from the rest of the participants.

Totally 26 handball and 22 football players completed the study. To be able to investigate possible differences between two groups, such as athletes mainly training indoor and athletes mainly training outdoor, at least 40 subjects had to be included in this study (20 in each of two groups), as described in section 3.6. Therefore, the number of athletes was sufficient to investigate possible differences between the handball and football players. A large study sample is also favorable to get a more accurate measure of the 25(OH)D level.

5.1.2. *The representativity of the sample*

We chose a female study population to investigate the vitamin D status of elite athletes since 25(OH)D levels can influence bone health. The prevalence of the Female Athlete Triad in female elite athletes is of great concern. Different aspects of the Female Athlete Triad have been evaluated for female football players in Norway [7], but the vitamin D status has not been investigated in relation to the Triad. Since vitamin D has known effects on bone health via its effect on calcium metabolism this can be an interesting aspect to consider.

Female top-level handball and football players were chosen to get knowledge of the vitamin D status of female elite athletes in the Oslo area. The two sports with most female participants in Norway are handball and football, and the top leagues for both sports are of high international level. We also wanted to investigate a possible difference in 25(OH)D level between athletes training mainly indoor (handball) and athletes training mainly outdoor (football). Therefore, these two sports were ideal for recruitment of many female elite athletes.

The age, weight, height and BMI of the athletes in the present study (Table 5) were similar to that found in other studies of female handball and football players in Norway [7;102;103], and similar to that found in studies from other countries including female athletes in ball sports [34;104;105].

In our study mean daily hours of training during the record period for the study population in total was 1.5 (0.52) hours. This is similar to findings for female Norwegian National Team football players [103], but lower than found in female Norwegian elite football and handball players [7;102]. Our study was performed in the end of the season for the football players and in the beginning of the season for the handball players, and this might have affected the number of training hours. Number of subjects in our study is similar to or higher than in other dietary surveys on athletes [34;103-106].

Taking the results from the other studies into account, it is likely that the results from the present study can be generalized for female elite athletes in ball sport in Norway. They cannot with certainty be generalized to the entire population of female elite athletes in Norway. However, the results can be used as an indication for expected findings in coming studies on vitamin D status in female elite athletes in Norway.

We also wanted to evaluate if our study sample was representative for female elite athletes in Norway for factors affecting sun exposure. A majority of the subjects were born in Norway (90%) (Table 6). Most of the subjects had a light pigmented skin type, most likely with low melanin production, and thus high vitamin D production in the skin (when sunlight is available), contributing to a higher 25(OH)D level than darker skinned people [107]. Thus, the athletes are likely to have a skin type representative for female elite athletes in Norway.

Initially two handball and two football teams from their respective top league were invited. Due to relatively low positive response, one more team of each sport was invited. Since only two handball teams in the top league were situated in the Oslo area, we chose to include a team highly placed in the 1st division instead of inviting a top league team with a longer travel to get to the Oslo area. The characteristics, 25(OH)D

levels and exposure factors in this team did not deviate from the other teams (data not shown), indicating that including one team not playing at the highest level did not have a great influence on the results.

There is always a risk that the participants are more conscious about their health and diet than persons not willing to participate. However, in this study, athletes less conscious about their health and diet could possibly also see the benefits of participating, since individual dietary feedback can lead to better sport performance.

5.2. *Methods*

5.2.1. *Blood sampling*

All blood samples were collected between October 5th and November 12th 2010. Since the subjects' samples were collected in a very short time interval the 25(OH)D levels can be compared between individuals, and seasonal fluctuation does not need to be considered a factor contributing to deviating 25(OH)D levels between the subjects.

As described in the introduction, Webb and Engelsen [57] have estimated that between mid-October until beginning of March, vitamin D cannot be produced from sunlight at 60°N. From this we can conclude that our blood samples from October/November were taken in the beginning of the "vitamin D winter".

Vieth [10] reviews that in temperate climates serum 25(OH)D levels rise and fall throughout the year in a pattern that parallels, with a delay of about two months, the intensity of UVB-light. In cross-sectional studies, especially from relatively elevated latitudes in North America, Europe and Asia, serum levels of 25(OH)D are maximal 30–60 days after peak sunlight exposure in the summer months [2]. Sunlight production of vitamin D is highest in June and July, and therefore we estimated maximum 25(OH)D level to occur in August/September for our study population, and this is also supported by other studies on Nordic and British populations [59;108-110].

The 25(OH)D level in the samples taken in October/November thus likely reflects sun exposure from August/September, and represents a 25(OH)D level slightly below the

maximum. A new blood sample will be taken in April 2011, since levels are thought to be at their lowest in March/April [59;63;110]. The results from the last blood sampling will not be assessed in this thesis due to the limited time of a master study.

Serum 25(OH)D concentration is the most valid estimate for determination of vitamin D status in humans [16-18]. However, it should be kept in mind that the analysis method for determination of 25(OH)D_3 used in this study has a relative SD of 20%.

We chose to set the cut-off value for sufficient 25(OH)D level at 80 nmol/l. There is, as described in the introduction, no consensus in the literature, but recent studies indicate either 75 nmol/l or 80 nmol/l to be levels of sufficient vitamin D. Several authors suggest that vitamin D deficiency or insufficiency should be defined as <80 nmol/l [34;49-52]. A similar study by Halliday et al [34] on American college athletes also used 80 nmol/l as cut-off for vitamin D sufficiency. We considered that the blood sampling in the present study is likely to represent just below peak values. Since the study was performed at a latitude as high as 60°N, the levels are likely to fall during winter, and we considered it favorable to choose the highest cut-off value for sufficiency supported by literature.

5.2.2. *Diet record*

All prospective diet records have potential errors as a result of subjects changing their diet during the record period or not include all they eat or drink in the record [111]. We wanted to get data both at group level and individual level (to be able to give the athletes feedback about their own results), and weighed food record was the method of choice as it is the most widely used and most accurate of dietary methods [111].

Weighed food record is a time consuming method and might be a burden for the participants. The weighing of each food item and drink at the time of consumption may not be convenient because of lack of time and patience [112]. For athletes, the number of recording days is a compromise between accuracy and compliance. When increasing the record period, accuracy may suffer due to reduced compliance and altered eating behavior to simplify the recording process [113]. Periods longer than

three to four days reduce compliance and accuracy and have a higher drop-out rate [114-116].

For athletes, studies have demonstrated good agreement between mean energy intake and mean energy expenditure when food intake was self-reported by normal-weight, self-selected, highly motivated volunteer subjects using weighed records [117]. The development of the four-day weighed diet record presented in this study is based on the fact that diet records over three to four days are the most widely used method for dietary assessment of athletes [112;118]. The four-day weighed diet record is the preferable method to assess the energy intake and dietary patterns of elite athletes at Olympiatoppen.

Studies on general population have found underreporting to be 20% in average in diet records [119]. It is generally recognized that athletes also probably underreport in diet records [114;120], and it has been estimated that athletes underreport by 10-30% in weighed records [112].

Since energy expenditure was not measured in our study, underreporting of energy intake cannot be estimated directly. Basal metabolic rate (BMR) was calculated for all athletes using Harris and Benedict's formula [121]. Physical activity level (PAL) was estimated by dividing energy intake (since energy expenditure was not measured) with calculated BMR for each individual. This resulted in a mean PAL of 1.6, with values ranging from 0.9 to 2.4. For adults from Nordic population groups, a PAL of 1.8 represents a sedentary work situation and regular leisure time activity [96]. Therefore, there must, to some extent, be underreporting among our athletes. This should be kept in mind when interpreting the results.

According to Burke et al [114] the recording accuracy in athletes improves when they get instructions about the method, and are motivated to report good data for the feedback on their own diet. In the present study, the subjects got written and oral instructions about the implementation of the record period. They were also explained

the advantages of an accurate record. Meeting the subjects in person instead of instructing participants over telephone is a strength of this study [100].

In order to limit drop-out rate and improve accuracy of the diet record, the record days did not have to be consecutive, to reduce the burden on the subjects. They could choose the days best fitting their own time schedule and could then avoid diet recording on travel days. How representative the chosen days were with respect to normal diet is always to be considered. One of the record days had to be a Saturday, since the energy intake has been found to be significantly higher on Saturdays and Sundays compared with the average of the weekday values [122]. The subjects were encouraged to perform the record within approximately two weeks after the instruction session to get as small time period for the data collection as possible.

When handing in the diet notebooks, the investigator looked it through and asked questions when there were ambiguities in the diet record, in order to limit potential recording errors. When needed, e-mails and phone calls were used in the coding process to solve questions about food types and amounts. The subjects answered a questionnaire (Appendix 5) to check the representativity of the diet record period. This was included to assess whether the subjects thought these days represented their normal diet or if there were something special these days that could have resulted in deviations in the dietary intake. Results from this questionnaire did not exclude any subjects in the final analyses.

The investigator did all the data coding. One person coding is ideal since this give the least error [123], as the procedures are performed in the same way throughout the coding. However, when the coding is not verified by a second person some errors might also occur. When a certain food was not found in the database, the food most similar was used. Amount of vitamin D in the product was considered the most important criterion. Some of the subjects used a Danish margarine, which is not supplemented with vitamin D and vitamin A like all Norwegian margarines. A

Norwegian margarine was used as data code, and vitamin D and vitamin A were subtracted from the margarine intake for these subjects afterwards.

Determining vitamin D content in foods is difficult because only small quantities are present, even in vitamin D-fortified foods, and analyses are difficult since many other compounds are extracted along with vitamin D [124]. However, a recent review describes that existing methods for analyzing the vitamin D content in foods can produce accurate results [125].

In our study, most calculations of energy and nutrient intakes were done on group level, and a four-day diet record might be representative for the mean vitamin D intake. However, calculations where the subjects' were ranked after dietary intakes were also performed, and individual feedback was given. A four-day weighed diet record is insufficient to measure an individuals' habitual vitamin D intake. Due to small amounts of vitamin D in the diet and large day-to-day variations, more days are thought to be needed to find the true vitamin D intake at individual level.

No studies have estimated the amount of days needed to estimate vitamin D intake, but results for other fat-soluble vitamins (which also are stored over time) can be used to evaluate the days needed for vitamin D. The days needed to rank subjects with a given level of accuracy were for adult women estimated to be 16 days for vitamin E and 21 days for vitamin A (retinol) [126].

Calcium intake previously has been found to associate positively with 25(OH)D level [77;127], and therefore calcium intake was also investigated in our study. For calcium, 5-8 days are estimated needed for adult women [126]. Therefore, the four record days in our study is likely to be quite representative for usual calcium intake both at group level and for ranking subjects within the group.

Athletes in energy balance will have the same micronutrient needs as the general population, except for some athletes that might have higher need due to losses of

nutrients in sweat and urine [128]. However, it is agreed that the general recommendations for intake of micronutrients take this into account and therefore also can apply to athletes [129;130]. It is also suggested that the dietary needs of athletes will be covered in the diet due to a higher energy intake as a result of training [129]. Therefore, general recommendations for vitamin D and calcium intakes can apply for our athlete population.

5.2.3. *Training record*

The athletes recorded their training load (number of sessions per day and duration) on the same days as the diet record. This was to characterize amount of training by the study sample, to investigate possible differences between the handball and the football players, and also to ensure that foods and drinks ingested under training were reported.

5.2.4. *Questionnaires*

The questionnaire variables were all self-reported, which can provide recall errors. However, all questionnaires were interviewer-administered to limit ambiguities. It has been widely recognized that there is an inverse relationship between the recall length of time and the accuracy of the reported estimates [131]. E.g. when considering ambient UV levels, increased error can be introduced when the subject reports at home after the event rather than at the place and time of exposure [132]. Retrospective questionnaires about outdoor activities during a specified time period are often used in exposure studies, as in ours. The questions are convenient for interpretation and generalization, but the reliability of such recall is unknown [132]. A common problem in cross-sectional studies is that recalled exposure data are biased by the subject's knowledge of their own status with respect to the disease or outcome under investigation [61]. In our study however, information about vitamin D status and exposure factors were not provided to the athletes before the study was ended.

Country of birth and parents' country of birth were asked for as measures of skin type (Appendix 6). Also, skin response to acute and long-term sun exposure, hair color and

eye color (Appendix 8) were used to provide information about skin type. However, due to few subjects in some of the categories these variables were not further assessed. Most of the subjects reported that they were not very easily burned and had no difficulty in tanning. This is not representative for skin type I or II (Caucasians) [57], and country of birth/parents' country of birth were thought to be better indicators of skin type in our study population. However, since most subjects reported Norway as own and parents' country of birth, this variable did not turn out to be of importance regarding differences in 25(OH)D levels.

A questionnaire about usual supplement use (Appendix 7) was included since four record days may not be sufficient to be representative for the subjects' habitual supplement use. The questionnaire might give a better estimate of supplement use over time than the diet record.

The questions about sun exposure and sun behavior factors (Appendix 8) have previously been validated [61]. Of the questions from Appendix 8, only training outdoor and indoor in summer have not been validated. The questions about daily hours in daylight in summer, and hours of training indoor and outdoor in summer were used to describe sunlight exposure in summer. The variable 'hours in daylight the last week' has in a previous study been transformed to 'UV-hours' [61], which was considered a better metric of sun exposure than using hours in daylight. This was not done in our study and can be a limitation of this variable.

None of the subjects reported going on a sun vacation after September and therefore this variable was not investigated. The variables residing only in Nordic countries in summer and use of tanning bed the last month did not describe frequency and amounts, and differences could be difficult to discover in our relatively small study population. Similar variables were used by Brustad et al [61], who however had a much larger study population.

5.3. *Results*

5.3.1. *Serum 25(OH)D level*

The mean 25(OH)D level found in our study was 99 nmol/l. Halliday et al [34] found higher 25(OH)D levels for athletes in fall, while lower levels have been found in summer/fall for athletes by Lethonen-Veromaa et al [93] and Maimoun et al [92] (Table 4).

The study by Halliday et al is similar to ours. The purpose of their study was to examine the 25(OH)D level of male and female college athletes during the university academic year, and to examine if their 25(OH)D level was related to dietary intake, training and lifestyle habits and/or body composition. Finally, they evaluated to which extent insufficient vitamin D status was linked with compromised bone density or increased risk for illness or inflammatory injuries. Bearing the differences in latitude (41.3°N vs 60°N) and time of blood sampling (September/October vs October/November) between the study by Halliday et al and ours in mind, it is still of interest to compare the results.

The mean 25(OH)D level (99 nmol/l) in our study population of was similar to the set cut-off value for optimal level of ≥100 nmol/l. Two of the subjects had levels of 190 nmol/l, increasing the mean substantially, and only 38% of our subjects had optimal levels. Halliday et al [34] found 75.6% to have optimal level (≥100 nmol/l) in fall, which is more than twice that found in our study. The study by Halliday et al was carried out at 41.3°N, a latitude where vitamin D synthesis equivalent to 25µg occurs within minutes of sun exposure in the period April to October [133]. In our study at 60°N, sun exposure required to produce 25µg vitamin D within minutes only occurs in mid-summer, while hours are needed to achieve this level of sun exposure from August [133]. This might be an explaining factor for more subjects with optimal level in the study by Halliday el al than in our study.

We found the percentage of subjects with insufficient levels (<80 nmol/l) to be 27%. This is more than twice the 12.2% found in fall in the study by Halliday et al [34]. However, a higher insufficiency rate was found in a whole year study on young Israeli athletes at latitude 31.8°N [90]. As much as 73% were found to have 25(OH)D levels

<75 nmol/l, even though most of the sampling was done in the summer months (May-October). The insufficiency rate was very high considering Israel being a sunny country. Constantini et al [90] discussed if diet could explain their findings. Dietary intake was however not recorded in the study, but low ferritin levels were found, and they used ferritin levels as a surrogate marker for diet quality. Constantini et al [90] found the 25(OH)D levels to correlate to both season and ferritin levels. They suggested that both inadequate sun exposure and low dietary vitamin D could explain the findings. The 25(OH)D levels found among our athletes might also be influenced by dietary as well as sun exposure factors, which is further discussed in section 5.3.2.

Since few studies on 25(OH)D levels of athletes have been carried out, we decided to compare our results with those of studies on non-athletic population groups, including females and Caucasians, performed at somewhat comparable latitudes. As described in Table 4, similar and lower 25(OH)D levels than in the athletes in our study have been found in summer/fall in studies on general populations. The studies from more northern latitudes found lower 25(OH)D levels than us, which could be an indication of the importance of sunlight as source for vitamin D. However, the studies from lower latitudes, which have higher sun exposure in fall [133], also found 25(OH)D levels lower than or similar to our study. Other factors than solar senit angle (season and latitude) are also of importance for skin synthesis of vitamin D, such as altitude, tanning bed use, sunscreen use, clothing and outdoor behavior [134].

Blood sampling was carried out in October/November in our study, when 25(OH)D levels are likely to be somewhat below maximum [59;108-110]. Halliday et al [34] also investigated wintertime levels, and found a decrease in mean 25(OH)D level of 46.2 nmol/l (38%) from the level in fall. As much as 63.3% of the subjects turned out to have insufficient levels in winter. The wintertime drop in the mean 25(OH)D level was found to be 29.0 nmol/l (46%) in young Finnish female athletes [93]. This study was performed at latitude 61°N, quite comparable to ours. The seasonal differences in 25(OH)D levels found in these studies can give an indication of the degree of wintertime drop to expect for our subjects.

However, in a study on Norwegian general population (69ºN) Brustad et al [60] concluded that the generally high dietary intake of vitamin D, especially in winter, masked largely the effect of seasonal variation in UV-exposure, and caused an atypical seasonal variation in 25(OH)D levels in this study population. If vitamin D intake for our athletes is not sufficient to maintain 25(OH)D levels during the winter, low sun exposure at this high latitude is likely to lead to a substantial wintertime drop.

In our study the handball players had significantly higher 25(OH)D levels than the football players (Table 7). This contradicts previous findings of outdoor athletes having higher 25(OH)D levels than indoor athletes. Halliday et al [34] found significantly higher 25(OH)D levels in outdoor than indoor athletes in fall among American college athletes (p=0.013). Constantini et al [90] found that in Israeli athletes the prevalence of 25(OH)D levels <75 nmol/l was higher in athletes performing indoor versus outdoor sports (80% vs 48%, p=0.002). In both studies, the higher level among outdoor than indoor athletes was proposed to be caused by a possible higher exposure to sun among the outdoor athletes.

In our study the ten highest 25(OH)D values were found in handball players (data not shown), leading to a substantial increase in the mean among handball players, even though some of the handball players had low levels. When grouping in insufficient and sufficient 25(OH)D levels, no difference between handball and football players was found. This can imply that there might not have been an actual difference in vitamin D status between the handball and the football players.

5.3.2. *Exposure factors - Effect of dietary intake, supplement use and sun behavior on 25(OH)D level*

Neither BMI nor age turned out to be associated with 25(OH)D levels in our study. As described by Burgaz et al [135] the narrow BMI range and age range may explain the absence of an association between serum 25(OH)D levels and BMI or age. The a lack of correlation between 25(OH)D levels and BMI in our study is thus most likely

explained by the small range in BMI in our athlete population (between 19.6 and 26.4 kg/m^2), as also found for athletes by Halliday et al [34].

The age ranged between 18 and 33 years in our study, and thus was below the age when the capacity of the skin to produce vitamin D is significantly reduced. A slight decrease in skin production of previtamin D_3 has been found in as young as 18 year old skin [66], but the decrease is not substantial for young persons as those in our study [66-68].

Energy and macronutrient intake

Mean energy intake for the subjects was 10.0 MJ in our study (Table 8), comparable to 10.9 MJ found in female Norwegian National team football players [103]. Similar levels of energy intake have been found in other studies on female athletes including ball sports [104;105;136;137].

Macronutrient intakes as percentages of energy intake (Table 8) were all within general Norwegian recommendations [96]. Carbohydrate intake was below Norwegian athlete recommendations, while all other macronutrients were within recommendations [130]. Compared to the study on female Norwegian National team football players [103], carbohydrate intake was clearly lower, fat intake somewhat lower and protein intake somewhat higher in our study.

Vitamin D intake

No association between vitamin D intake (including or excluding supplements) and 25(OH)D levels were found, supported by findings by Halliday et al [34]. As mentioned in the introduction, vitamin D intake has previously been found to have positive correlation to 25(OH)D levels in subjects with low sun exposure [61;70;71]. Halliday et al [34], state that lack of relation between the 25(OH)D level and the estimated vitamin D intake from food (excluding supplements) is not surprising given that vitamin D is limited in the food supply. Since fortification of foods in Norway is less than in the US [69], this statement about the American diet can be even more

applicable for Norwegians. However, including supplements there is also a lack of a correlation between 25(OH)D levels and vitamin D intake. This may be explained by the fact that most circulating 25(OH)D is thought to originate from sunlight exposure rather than from dietary sources [32;54].

Including supplements the vitamin D intake was 4.5 µg/day (Table 8). This was higher than the 3.8 µg/day found for female Norwegian National team football players [103]. Jorde and Bønaa reported a vitamin D intake of 5.9 µg including supplements among Norwegian women [73]. Excluding supplements, the vitamin D intake from food sources in our study was 3.9 µg/day (Table 8), similar to 3.9 µg and 4.0 µg found for Norwegian women in the nationwide Norkost 1993-94 and 1997 dietary surveys [138].

Halliday et al [34] found daily intake of vitamin D among American college athletes in fall to be 6.1 µg (242 IU) excluding supplements and 13.8 µg (553 IU) including supplements. These values are much higher than found for our athlete group. However, the dietary intake of vitamin D in US is generally higher than in Norway due to less restrictive fortification [69]. Other studies on athletes outside of Norway have found dietary vitamin D intakes in the range of 2.4-4.6 µg/day for females [93;139-141], and thus similar to that found in our study.

As many as 35 (73%) of the athletes had a dietary vitamin D intake <7.5 µg, and ten (21%) of the athletes had a vitamin D intake below the lower limit of 2.5 µg/day. This is quite similar to 79% and 12%, respectively, found for top-level endurance athletes in Norway [97]. It must, however, be taken into account that our four-day diet record might not be sufficient when investigating vitamin D intakes on an individual level.

Vitamin D sources

The three largest contributors to vitamin D from food were fish, egg and butter/margarine, supported by findings for top-level endurance athletes in Norway [97] and Norwegian women [61;73]. The three main sources contributed to 76% of the

dietary intake of vitamin D excluding supplements, which points out that there are few food sources with substantially amounts of vitamin D.

Calcium intake

The subjects with total calcium intake ≥800 mg/day had higher 25(OH)D level than the subjects with total calcium intake <800 mg/day. However, calcium intake per ce did not correlate to 25(OH)D levels (Table 11). Lips [77] concluded that the appropriate 25(OH)D level can be influenced by dietary calcium, where a low calcium intake increases PTH and leads to an increase in the turnover of vitamin D metabolites. The 25(OH)D level may therefore be somewhat higher with a high calcium intake and lower with a very low calcium intake.

Average calcium intake including supplements was found to be 990 mg/day (Table 8). The intake of calcium supplements was low, leaving calcium intake excluding supplements almost as high (970 mg/day). The intake in our study population was similar to 989 mg/day found for female Norwegian National team football players [103]. Two of the subjects had a calcium intake below the lower limit of 400mg/day, which can be explained partly by a low energy intake.

The prevalence of the Female Athlete Triad in female elite athletes is of great concern also in Norway. Sundgot-Borgen and Torstveit [7] found in studies on female Norwegian elite athletes that although less common than in other sports, also football players are dieting and experiencing eating disorders, menstrual dysfunction and stress fractures. Of female Norwegian football and handball players 9.3% and 18.8%, respectively, reported to have menstrual dysfunction [7]. Whether a daily calcium intake of 1500 mg should be recommended for athletes with menstrual dysfunction has been debated [142]. Along with the statement that high calcium intake increase 25(OH)D level [77], this raises the question whether the calcium intake for our study population actually was sufficient or not.

Supplement use

In our study 23 (48%) of the subjects reported using supplements regularly. Other Norwegian studies have found the use of supplements to be higher in endurance athletes than other athletes [106;143-145]. Of athletes in technical sports, 68% recorded to use supplements [106]. This is also higher than observed in our study.

The subjects using vitamin D supplements in our study reported that they supplemented regularly throughout the year. Of the 12 subjects reporting to use vitamin D supplements, 11 subjects took a vitamin D supplement during the record days, and the amount of vitamin D supplements used these four days may be representative for the normal use. As one subject had a very high intake of vitamin D from supplements, analyses were also performed without this person, which was outlier, however vitamin D intake between the subjects using and not using a vitamin D supplement still was different at the same significance level ($p<0.001$).

We found no correlation between reported intake of a vitamin D supplement and 25(OH)D levels. Other factors, like sun exposure, might be of greater importance for 25(OH)D levels, and possibly mask the effect of vitamin D supplements on 25(OH)D levels. Another explanation for the lack of correlation can be that the amount of vitamin D from supplementation was not high enough to increase the 25(OH)D levels. In a study of Finnish young female athletes the 10 µg vitamin D supplementation did not significantly succeed in reducing the prevalence of vitamin D deficiency [93]. Reviewing the vitamin D status in athletes Willis et al [35] state that the 10 µg vitamin D in a multivitamin supplement is likely not sufficient.

Sun behavior

The variables we used for evaluation of UV exposure, except for training outdoor and indoor in summer, were all based on the questionnaire used in the Norwegian Women and Cancer Study (NOWAC) of female melanoma patients in northern Norway (latitude 65-71ºN) by Brustad et al [61]. According to their findings, we could expect hours in daylight last week and tanning bed use to be positively associated with

25(OH)D levels, and residing only in Nordic countries in summer to have a negative association with 25(OH)D levels.

Figure 6 presents the associations between sun exposure factors and 25(OH)D level found in the present study.

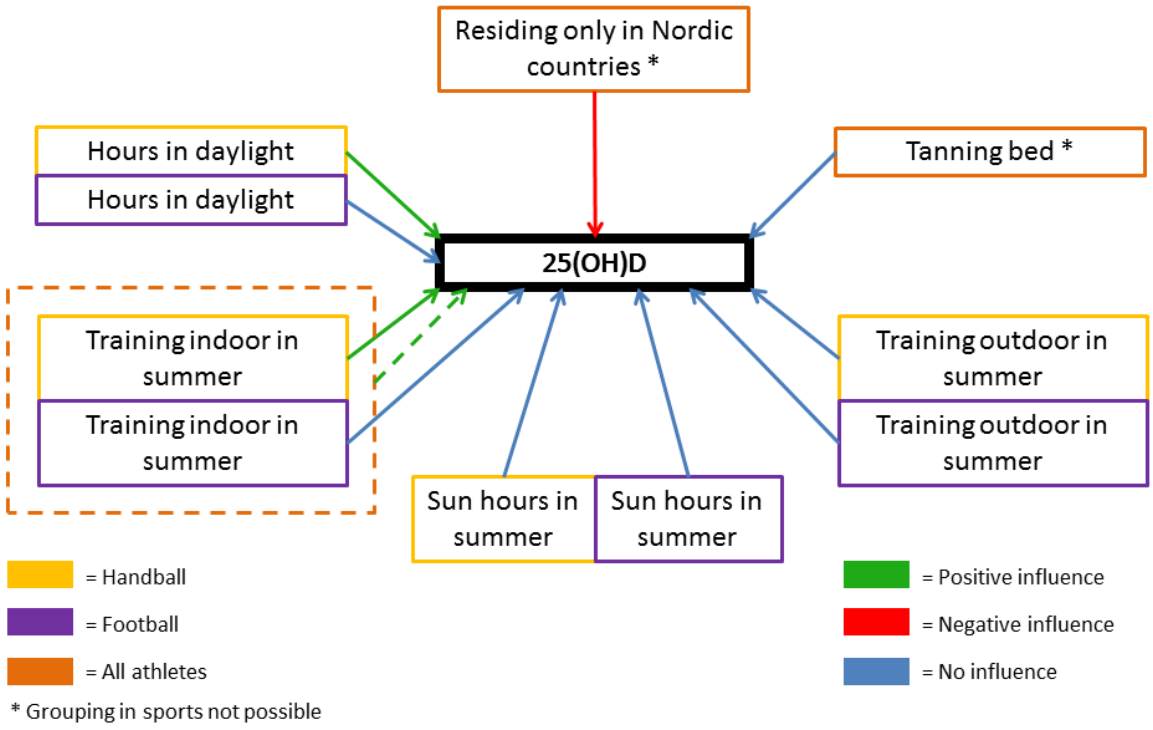

Figure 6: Associations between sun exposure factors and 25(OH)D level.

In the present study hours in daylight the week prior to blood sampling did not correlate to 25(OH)D levels for the athletes in total (Table 11, Figure 6). For the handball players a significant positive correlation was found, while no correlation was found for the football players. However, the number of daily hours in daylight the last week prior to blood sampling was significantly higher for football players than handball players (Table 10). Thus, hours in daylight last week did not seem to be an important factor for the 25(OH)D levels in our study.

Reasons for hours in daylight last week not being associated with 25(OH)D levels for the athletes in total could be that the hours in daylight last week was found, by Brustad et al [61], to be a less accurate measure of sun exposure than estimated UV-hours. They also found sun vacation and use of tanning beds to be more important for 25(OH)D level than UV-hours. It should also be kept in mind that the last week, being in October/November in our study, may not be representative for the sun exposure the last months. The reason for the positive correlation found for the handball players remains unknown.

Sun hours in summer did not correlate to 25(OH)D levels (Table 11, Figure 6), neither for the athletes in total, nor for the handball and football players. Halliday et al [34] investigated reported leisure time spent outdoor in fall, winter and spring, and did not find correlation to 25(OH)D levels at any time point.

Serum 25(OH)D levels were significantly lower (Table 12, Figure 6) for the subjects residing only in Nordic countries in summer than for the subjects going outside of Nordic countries in summer. This was the only variable found to explain some of the variation in 25(OH)D levels.

Brustad et al [61] investigated the variable residing only in northern Norway during summer and found that it significantly predicted 25(OH)D levels in November-February. That extent of sun exposure in summer still predicted 25(OH)D levels in winter is an indication of a long-term effect of sunlight. If the increased sun exposure among our athletes going outside of Nordic countries also affects their 25(OH)D levels in winter, like Brustad et al found, will be investigated when new blood samples are taken in April 2011.

Number of handball players residing only in Nordic countries in summer prior to blood sampling was significantly lower than the number of football players. The fact that the handball players travelled more south in summer can be an important reason for the higher 25(OH)D level found in the handball than in the football players.

No change in associations between exposure factors and 25(OH)D level was found when sub-analyses for the subjects residing only in Nordic countries and not were performed. Also, no significant differences with respect to dietary intakes, supplement use and sun exposure were found between the subjects residing only in Nordic countries and not. However, the total vitamin D intake for all athletes appeared to be slightly higher for those residing only in Nordic countries (Table 13), and this could reduce the effect of going outside of Nordic countries in summer on 25(OH)D levels.

Serum 25(OH)D levels were not significantly different between the subjects using tanning bed the last month and those not (Table 12, Figure 6), even though 25(OH)D levels among those using tanning bed appeared to be slightly higher. Brustad et al [61] found tanning bed use to be strongly associated to 25(OH)D levels ($p<0.0001$). This study had a much larger study sample. The lack of correlation found in our study may partly be due to a too low number of subjects for this variable. Also, only 13 subjects reported to use tanning bed the last month, and frequency and duration were not investigated.

Training hours outdoor in summer did not correlate to 25(OH)D levels in the present study. Unexpectedly we found training hours indoor in summer to have a significant positive correlation to 25(OH)D levels for all athletes (Table 11, Figure 6). When grouping into sports, there was also a correlation for the handball players, but not for the football players (who had little indoor training). This could be explained by the significantly higher 25(OH)D levels found in the handball players, who trained significantly more indoor, but resided significantly less in Nordic countries in summer than the football players. We wanted to perform sub-analyses to investigate if the variable residing only in Nordic countries in summer could explain the correlation between training indoor in summer and 25(OH)D levels, but the number of subjects in each group were insufficient to be able to draw conclusions.

6. Conclusions

In this study, of female elite athletes living at high latitude, the average 25(OH)D level in fall was similar to optimal level, and there were large inter-individual differences. Vitamin D insufficiency was found in one out of four of the athletes. Going outside of Nordic countries in summer seemed to be the factor of most influence on 25(OH)D levels in the athletes. Since the subjects live at high latitude and since vitamin D intake were below recommended level for ¾ of the subjects, their 25(OH)D levels are likely to decrease during winter. Hence, it needs to be debated if routine screening for 25(OH)D levels in female elite athletes should be recommended.

Unlike other studies of athletes we did not find lower 25(OH)D levels in indoor than outdoor athletes. Less subjects residing only in Nordic countries in summer may be the reason for a higher 25(OH)D level found among the handball than the football players. No other exposure factors could explain the variation in 25(OH)D levels in our study.

7. Future work in the field

This study is one of the first to investigate vitamin D status in elite athletes in Norway. The main purposes were to measure the 25(OH)D level in our female elite athlete study population, and to investigate possible factors important for the 25(OH)D level. This limited study can be considered as a pilot study for coming studies on vitamin D status in female elite athletes in Norway. These studies should, however, include a larger study sample, and the variables for sun exposure used in the present study should be improved.

As a continuation of this study, the subjects are asked to take a new blood sample in April 2011, to investigate the wintertime drop in 25(OH)D levels. In future studies, possible predictors of 25(OH)D levels from diet, supplements and sun exposure should be included, as in our study. We did not investigate oral contraceptive use. This has in previous studies been found to predict 25(OH)D levels [146;147], and this could be an important factor to investigate in future studies on vitamin D status in female athletes.

More studies are needed to establish the vitamin D status of Norwegian female athletes, and especially wintertime levels. The impact of vitamin D insufficiency on athletic performance and health is yet to be established.

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9. Appendices

Appendix 1: Example of written feedback to a subject

Kjære deltaker,

Tusen takk for flott innsats i forskningsprosjektet *"Vitamin D status among female handball and football elite athletes in Norway at latitude 60ºN".*

Vedlagt finner du en vurdering av kostholdet ditt, basert på de fire dagene du veide og registrerte inntaket ditt. Blodprøvesvaret for vitamin D nivået ditt står i tabell 2 på side 1. På siste side finner du anbefalinger når det gjelder kostråd i forbindelse med trening.

Hvis du har noen spørsmål vedrørende tilbakemeldingen eller ønsker å få en nærmere gjennomgang av resultatene dine, send mail til k.l.jonvik@studmed.uio.no.

Som nevnt tidligere ønsker Olympiatoppen å ta en ny blodprøve for å se på endring i vitamin D-nivå etter vinteren. Vitamin D-nivået er ofte lavere etter vinteren grunnet lite sollys. Du vil motta en forespørsel om deltakelse på mail fra Christine Helle, fagansvarlig for ernæringsavdelingen på Olympiatoppen. Det er helt frivillig, men vi oppfordrer deg til å delta.

Vennlig hilsen

Kristin Lundanes Jonvik

Masterstudent i klinisk ernæring, Universitetet i Oslo.

Tilbakemelding NN

Periode:

Tabell 1: Inntak av næringsstoffer

	Ditt daglige inntak		Anbefalt daglig inntak
	Uten kosttilskudd	Med kosttilskudd	
Energi (kcal) **Energi (kcal per kg)**		-	
Protein (%) **Protein (g per kg)**		-	12-20 1,2-1,6
Fett (%)		-	20-35
Karbohydrat (%) **- sukker (%)** **Karbohydrat (g per kg)**		-	50-70 < 10 5-8
Kostfiber (g)		-	35
Alkohol (%)		-	0
Vitamin D (µg)		-	7,5
Kalsium (mg)		-	800
Jern (mg)		-	15
Vitamin C (mg)		-	75

Forklaring til anbefalingene: Anbefalingene gjelder for kvinnelige idrettsutøvere i lagidrett. Anbefalingene for karbohydrat og protein uttrykt i gram pr kg kroppsvekt angir ditt absolutte behov. Anbefalingene for karbohydrat, protein og fett uttrykt i prosent viser hvor mye de tre næringsstoffene bør bidra med av ditt totale energiinntak, og angir den ideelle sammensetningen av kostholdet til idrettsutøvere. Det er det absolutte behovet det er viktigst at du får dekket. Anbefalingene for kostfiber, vitamin D, kalsium, jern, vitamin A og vitamin C er absoluttverdier og gjelder for kvinner.

Tabell 2: Vitamin D-status (blodprøve)

	Din verdi	Anbefalt verdi
25(OH)D (nmol/l)		50-150

Vurdering av kostholdet ditt

Proteininntak:

Av tabell 1 ser du at du får i deg nok proteiner gjennom den kosten du spiser. Rene fileter av kjøtt, fisk og kylling er bedre proteinkilder enn pølser, salami og kjøttdeig (blandingsprodukter). Melk, yoghurt, egg og ost er også gode proteinkilder.

Karbohydratinntak:

Karbohydratinntaket ditt er lavt, noe som også fører til at du får i deg for lite kostfiber. Du kan med fordel øke andelen karbohydratrike matvarer i mange av måltidene. Spis brød i stedet for knekkebrød, spis brød til salat, spis mer pasta, ris eller potet i forhold til kjøtt eller fisk til middag. Det er en god regel å spise minst 2 måltider med brød og/eller korn hver dag. Bruk også brød, korn, yoghurt, smoothie, frukt og grønnsaker som mellommåltid.

Inntak av vitamin D:

Vitamin D inntaket ditt, som var hovedfokuset mitt i dette prosjektet, var ... under kostregistreringen. Det er ikke så mange kilder til vitamin D i matvarer, men fet fisk er en god kilde. I tillegg er ekstra-

lettmelk, margarin og smør tilsatt vitamin D. Tran er den største kilden til vitamin D i norsk kosthold, og 5ml (en barneskje) daglig er tilstrekkelig for å nå anbefalt inntak for vitamin D.

Inntak av jern, kalsium og vitamin C:

Inntaket ditt av jern og kalsium er litt lavt. Gode jernkilder er grove kornprodukter og leverpostei, mens melkeprodukter og ost er gode kalsiumkilder. Vitamin C inntaket ditt er veldig bra.

Kostråd i forhold til treningsøktene:

Det er viktig at du spiser nok før trening slik at du er i energibalanse på hele treningsøkten. Et karbohydratrikt måltid 1-3 timer før økten starter vil medføre en siste fylling av glykogenlagrene og regulering av blodsukkeret. Før økter med styrketrening eller lange utholdenhetsøkter bør du ha et bra proteininntak i tillegg til karbohydratinntaket for å sikre optimal proteinomsetning.

Idrettsutøvere anbefales å drikke på alle økter som varer mer enn 30 minutter. Det er da viktig å drikke så mye at du ikke har et væsketap som overstiger 2% av kroppsvekten. Du bør drikke 5-8 dl per time trening. I varmt klima kan det være nødvendig å drikke mer.

Karbohydratinntak under trening vil øke arbeidskapasiteten din dersom du tømmer glykogenlagrene dine og/eller får lavt blodsukker i løpet av økten. For deg vil dette sannsynligvis være treningsøkter som varer mer enn 60 når det er høy intensitet og mer enn 90-120 min når det er lav intensitet. Anbefalingen for slike økter er å innta 30-60 gram karbohydrat per time trening i form av en sportsdrikke med 4-6% karbohydrat (tilsvarer 4-6 gram per dl vann). Du kan også få dette karbohydratinntaket fra mat med høy glykemisk indeks (energibar, moden banan, rosiner, loff med syltetøy etc), men pass da på at du tygger maten godt og drikker vann til. Energibarer gir 20-40 gram karbohydrat (se på pakken), 1 banan gir 20-25 gram, 1 dl rosiner gir 40 gram og 1 skive loff med syltetøy gir 20-25 gram.

Etter alle treningsøkter er det viktig å innta væske, karbohydrat og protein for at restitusjonsprosessene skal bli optimale. Du skal erstatte væsketapet ditt med 150% av væsketapet du har hatt i løpet av treningen. Det betyr at mengden du trenger vil avhenge av hvor mye du har drukket under økten. Prøv uansett å drikke minst 5 dl direkte etter økten og fortsette å drikke til du har tisset 2 ganger. Etter lange økter og i varmt klima må du sannsynligvis drikke vesentlig mer. Så bør du innta 50 gram karbohydrat innen ½ time etter alle økter, dette er spesielt viktig etter harde økter. Det kan du gjøre ved å innta karbohydratrike sportsprodukter/matvarer eller ved å spise et måltid med karbohydratrik mat.

Eksempler på karbohydratrike matvarer som gir ca. 50 gram karbohydrat er: 1,5 energibarer, 2 bananer, 1-1,5 dl rosiner, 1,5 rosinboller, 2 skiver loff med syltetøy. Eksempler på et måltid som inneholder ca 50g karbohydrat er: a) 1-2 brødskiver med syltetøy og 1 glass melk; b) 2 brødskiver med proteinrikt pålegg (ost, fisk, kylling, kjøtt, egg) og 1 glass juice til; c) 1 dl kornblanding med 2 dl fruktyoghurt eller 2 dl melk og 1 ss syltetøy; d) varmrett med 150 gram pasta, ris eller poteter, 100g grønnsaker og litt fisk, kylling, kjøtt eller egg.

Etter restitusjonsinntaket bør du spise et blandet måltid som gir både karbohydrat, protein og fett innen 2 timer etter avsluttet økt. Dette kan være brødmat, kornblanding, grøt eller varme retter.

Hvis du har noen spørsmål eller ønsker ytterligere veiledning er det bare å ta kontakt.

Lykke til!

Med vennlig hilsen,
Kristin Lundanes Jonvik

Appendix 2: Invitation letters

Forespørsel om deltakelse i forskningsprosjektet

"Måling av vitamin D-status hos kvinnelige toppidrettsutøvere i fotball og håndball i Norge"

Bakgrunn og hensikt

Dette er et spørsmål til deg om å delta i en forskningsstudie i feltet idrettsernæring. Formålet med denne studien er å måle vitamin D-status hos kvinnelige toppidrettsutøvere i Norge. Vitamin D kan være en faktor for prestasjon og helse, og vi ønsker å vurdere om det er et grunnlag for å screene kvinnelige toppidrettsutøvere for vitamin D-status. I tillegg ønsker vi å finne faktorer som påvirker utøvernes vitamin D-status.

For å gjennomføre studien trenger vi 40-60 frivillige kvinnelige toppidrettsutøvere som spiller henholdsvis fotball i Toppserien og håndball i Postenligaen. De må være over 18 år, og være friske og skadefrie i registreringsperioden. Derfor henvender vi oss til deg.

Studien er en masteroppgave i klinisk ernæring ved Universitetet i Oslo, og utføres i samarbeid med Olympiatoppens ernæringsavdeling. Den skal ledes av masterstudent Kristin Lundanes Jonvik, med veiledning fra ernæringsfysiolog Cand. Scient. Christine Helle, fagansvarlig ved Olympiatoppens ernæringsavdeling.

Hva innebærer studien?

Deltakerne i studien vil gjennomføre en 4-dagers veid kostregistrering i oktober/november 2010, og fylle ut spørreskjema om henholdsvis kosttilskudd og soleksponering/solvaner. Registreringsskjema og matvekt for å gjennomføre kostregistreringen vil bli delt ut til hver deltaker. I tillegg skal det tas to blodprøver av hver deltaker, det vil skje på Olympiatoppens helseavdeling. Den første blodprøven tas i oktober/november 2010 og den andre tas i mars 2011.

Hva kreves av deg?
Deltakerne møter opp ved Olympiatoppens lokaler på Sognsvann tre ganger. Første gangen er i forkant av studien til en veiledning i hvordan kostregistreringen skal utføres, da får du også låne en matvekt. Andre gangen er for første blodprøvetaking, i tillegg til innlevering av kostregistreringen og matvekten. Da vil det også bli spurt enkle spørsmål om bruk av kosttilskudd og solvaner. I mars vil deltakerne innkalles på nytt, kun for å ta en ny blodprøve.

Hva får du igjen for å bli med?
Fordelen med å delta i denne studien er at du får en grundig vurdering av kostholdet ditt. Du vil også få tilbud om en individuell veiledningstime hvis du ønsker det. I kostholdsvurderingen vil vi spesielt fokusere på faktorer som påvirker restitusjon og prestasjon, og se om du imøtekommer anbefalingene for din idrett. I tillegg får du vite din vitamin D-status.

Hva skjer med informasjonen om deg?
Informasjonen som registreres om deg, skal kun brukes slik som beskrevet i hensikten med studien. Alle opplysningene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. Det er bare en kode som knytter deg til dine resultater. Det er kun autorisert personell knyttet til prosjektet som har adgang til navnelisten, og som kan finne tilbake til deg. Andre personer kan dermed ikke se dine resultater, og det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres. Prosjektet er godkjent av Regionale komiteer for medisinsk og helsefaglig forskningsetikk.

Frivillig deltakelse
Det er frivillig å delta i studien. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke til å delta i studien. Du kan også trekke deg underveis i studien hvis du av en eller annen grunn ikke ønsker å fullføre.

Dersom du ønsker å delta, ber vi deg undertegne samtykkeerklæringen nederst på arket og sende den til oss innen mandag 6. september. Bruk vedlagte frankerte konvolutt. Hvis du ikke ønsker å delta, vil vi gjerne at du likevel krysser av for dette under og returnerer svarslippen i vedlagte konvolutt. Dermed slipper vi å purre på de som ikke vil delta.

Dersom du har spørsmål til studien, kan du kontakte Kristin Lundanes Jonvik på telefon 419 065 60.

Vennlig hilsen

Kristin Lundanes Jonvik
Masterstudent klinisk ernæring
Universitetet i Oslo

Christine Helle
Ernæringsfysiolog Cand. Scient.
Fagansvarlig ernæring
Olympiatoppen

✂----------

☐ Jeg ønsker å delta i undersøkelsen "Måling av vitamin D-status hos kvinnelige toppidrettsutøvere i fotball og håndball i Norge"

☐ Jeg ønsker ikke å delta i undersøkelsen "Måling av vitamin D-status hos kvinnelige toppidrettsutøvere i fotball og håndball i Norge"

Navn:……

Klubb:……..

Tlf. nr.:…….

E-post:…….

Samtykke til deltakelse i studien

Jeg er villig til å delta i studien

---------- ----------
Dato Signert av prosjektdeltaker

Jeg bekrefter å ha gitt informasjon om studien

---------- ----------
Dato Signert av prosjektmedarbeider, rolle i studien

Appendix 3: Approval from Regional Committee for Research Ethics

UNIVERSITETET I OSLO
DET MEDISINSKE FAKULTET

Fagansvarlig ernæringsfysiolog Christine Helle
Olympiatoppen
Pb. 4403 Ullevål Stadion
0806 Oslo

Regional komité for medisinsk og helsefaglig forskningsetikk Sør-Øst A (REK Sør-Øst A)
Postboks 1130 Blindern
NO-0318 Oslo

Telefon: 22 84 46 66

E-post: jorgen.hardang@medisin.uio.no
Nettadresse: http://helseforskning.etikkom.no

Dato: 21.09.2010
Deres ref.:
Vår ref.: 2010/1351a

2010/1351a Måling av vitamin D-status hos kvinnelige toppidrettsutøvere i Norge

Vi viser til e-post av 16.9.2010.

Av tilbakemelding på e-post framgår det at det biologiske materiale skal destrueres umiddelbart etter analyse. Det er derfor ikke noen grunn til å opprette en forskningsbiobank i dette prosjektet. Komiteen har dermed fått avklaring på de spørsmål som ble stilt og prosjektet kan godkjennes.

Vedtak:

Komiteen godkjenner at prosjektet gjennomføres i samsvar med det som framgår av søknaden og tilbakemelding på komiteens merknader.

Dersom det skal gjøres endringer i prosjektet i forhold til de opplysninger som er gitt i søknaden, må prosjektleder sende endringsmelding til REK.

Forskningsprosjektets data skal oppbevares forsvarlig, se personopplysningsforskriften kapittel 2, og Helsedirektoratets veileder for «Personvern og informasjonssikkerhet i forskningsprosjekter innenfor helse- og omsorgssektoren». Personidentifiserbare data slettes straks det ikke lenger er behov for dem og senest ved prosjektets avslutning.

Godkjenningen gjelder til 17.6.2011. Prosjektet skal sende sluttmelding på eget skjema, se helseforskningsloven § 12, senest et halvt år etter prosjektslutt.

Vennligst oppgi vårt saksnummer/referansenummer i korrespondansen.

Med vennlig hilsen

Gunnar Nicolaysen (sign)
Professor dr. med
Komitéleder

Jørgen Hardang
Komitésekretær

Kopi: Olympiatoppen ved øverste administrative ledelse

Appendix 4: The record notebook

Vitamin D-status hos kvinnelige håndball- og fotballspillere

Forskningsprosjekt ved UiO og Olympiatoppen

Id.nr. □□□□

Veiledning for kostregistreringen (gule ark)

- Du skal registrere kostholdet ditt i 4 dager (3 ukedager + 1 lørdag)
- Du skal også registrere treningen din i de 4 dagene
- Prøv å unngå at kostregistreringen forandrer matvanene dine - **spis slik du vanligvis gjør**!
- Skriv ned alt du spiser og drikker, også evt. kosttilskudd
- Skriv ned evt. væskeinntak og matinntak under trening på sidene for treningsregistreringen

- Start med det første måltidet den dagen registreringen begynner. Fyll inn alle måltidene du spise, både hoved- og mellommåltidene. Bruk en side per måltid. For hvert måltid skal følgende skrives ned:
  1) Klokkeslett
  2) Navnet på matvaren eller retten → gi flest mulig opplysninger
     - f.eks. Birkebeinerbrød, Norvegia helfet ost, Nora jordbærsyltetøy, lett melk, 15 kr Freia melkesjokolade
     - evt. oppskrift på hjemmelagete retter (skriv oppskriften bak på arket)
     - evt. hvordan retten er tilberedt (kokt, stekt ect.)

NB! Jo flere opplysninger du gir, jo riktigere blir beregningene. Se på matvareemballasjen når du skal notere navnet. Hvis det er spesielle matvarer, kan du ta vare på emballasjen og legge den med.

3) Mengde av matvaren eller retten
   → oppgi mengde i gram hvis du har vekt
   → oppgi mengde i husholdningsmål hvis du ikke har vekt
      - antall, stykker, spiseskje, teskje, glass, kopp, dl

Innholdet i glass, kopper og flasker som du bruker ofte, er det nok å veie en gang. Denne vekten oppgis hver gang du drikker av disse glassene eller koppene. Disse målene kan du føre opp på den siste siden i veiledningen.

Hvis du spiser hjemmelagete retter (hjembakt brød, havregrøt, vafler, kjøttsaus til spagetti, fiskegrateng etc.), må du skrive opp oppskriften i grove trekk. Du kan notere oppskrifter på baksiden av hvert ark. Legg gjerne med innholdsdeklarasjonen på kjøpte retter.

Måltider som spises borte. Dersom du i løpet av registreringsperioden spiser andre steder enn hjemme og det ikke lar seg gjøre å veie maten, skriv ned det du spiser så nøye som mulig og oppgi mengdene i husholdningsmål (dvs. antall, stykker, spiseskjeer, teskjeer, glass, kaffekopp etc.).

Porsjonstørrelser og stykkvekter av sjokolade etc.. Hvis du kjøper deg en sjokolade, ispinne e.l., trenger du ikke veie den fordi jeg har lister på vektene til disse vanlige matvarene. I stedet for vekt må du notere antall, f.eks. 1 kroneis m/ sjokolade Diplomis, 1 liten pose Maarud peanøtter, 1/2 pose Nidar halslinser.

NB! Du skal ikke fylle inn der det står Kode Mengde, dette gjøres av prosjektansvarlig.

Veiing

- Brødskiver: Nullstill vekten. Sett asjetten på vekten. Nullstill. Legg på brødskiven, les av og noter hva den veier. Nullstill. Smør på brødskiven, legg den på vekten igjen, les og noter. Nullstill. Legg på pålegg, les av og noter.
- Kornblanding: Sett tallerkenen på vekten. Nullstill. Ha i kornblandingen, les av og noter vekten. Nullstill. Ha i syltetøy/sukker etc., les av og noter vekten. Nullstill. Ha i melk/yoghurt etc., les av og noter vekten.
- Middag: Vei når retten er ferdig tilberedt (etter at maten er kokt, stekt etc.). Sett tallerkenen på vekta. Nullstill. Vei så en og en ting om gangen. Les av og noter vekten, og nullstill mellom hver gang du legger på noe nytt.
- Sammenkokte retter: Veies i ett (f.eks.fiskegrateng, gryterett o.l.)

NB!

- Ved å nullstille mellom hver gjenstand slipper du å trekke fra.
- Vent på nullen før du evt. tar asjetten av.
- Vekten veier ikke mengder på 2g eller mindre. Noter alle matvarer selv om de ikke gir noe utslag på vekta, slik som f.eks. minimalt med margarin på brødskiva, en agurkskive til pynt etc.
- Bein (f.eks. på kotelett), skinn (f.eks. på fiskeskiver), skall (f.eks. på banan) og annet som ikke er spiselig, veies for seg etterpå og trekkes fra vekten på den totale matvaren. Du kan evt. fjerne det før du veier. Hvis du ikke har anledning til å veie det før eller etter, husk alltid å notere at vekten på matvaren inkluderer skall, bein ect.
- Eventuelle rester på tallerkenen veies og trekkes fra vekten målt for hver matvare.

Veiledning for treningsregistreringen (grønne ark)
Her skal du registrere de treningsøktene du har i løpet av en dag. Ett ark per økt. Skriv ned type trening, intensitet (sone), varighet på økta og om du trente ute eller inne. I tillegg skal du registrere det du eventuelt inntar av væske og mat under økta. Beskriv type og oppgi mengde i ml eller gram. Hvis du ikke har vekt tilgjengelig, oppgi mengde i husholdningsmål (f.eks. 2 måleskjeer Maxim energidrikk i 750ml vann, 1 banan ect.)

SKRIV TYDELIG!

Ring eller mail meg hvis du har noen spørsmål; *# 419 06 560 eller k.l.jonvik@studmed.uio.no*

Lykke til!

Kristin Lundanes Jonvik
Masterstudent i klinisk ernæring
Universitetet i Oslo

Innholdet i glass, kopper og flasker brukt i registreringen. (NB Det er kun innholdet som skal veies!)

Glass …… gram Kopp …… gram Flaske……gram

Konsumdata (skrives på K-linjene i KBS)

Løpedagid skriv dagnr _____ **Ukedag** _____ skriv mandag = 1, tirsdag=2 osv_____

Måltidstype_____ skriv måltidskode _____

Vanlig dag (ja=1, nei=2) _____ **Klokkeslett** skriv hel time_____ **Spisested** _____

Måltidsdata (skriv kode og mengde atskilt av mellomrom på M-linjene i KBS IKKE FYLL INN I GRÅTT FELT! (til koding)

Matvare/rett – beskriv sammensetning og mengde i gram	**Kode**	**Mengde, g**

TRENINGSREGISTRERING **Dag 1**

NB! Skriv ned væskeinntak og matinntak (mengde og type) hvis du bruker det <u>under</u> trening

Økt 1 **Kl. ……..……..**

Type trening og intensitet **Varighet**

……..… **…….**

……..… **…….**

Trente du ute i dagslys eller innendørs?
……..

Type væske-mat inntak ***Mengde***

……..… **…….**

……..… **…….**

Kommentarer:

……
……...

Appendix 5: Questionnaire for control of the diet and training records

OPPLYSNINGER OM KOSTREGISTRERINGSPERIODEN

1. Har du i perioden du har registrert kostholdet hatt et "vanlig" kosthold?

☐ Ja ☐ Nei

Hvis nei, hva er endret i forhold til det kostholdet du vanligvis har?

- spist mer/flere måltider enn vanlig ☐
- spist mindre/færre måltider enn vanlig ☐
- mer bevisst i forhold til valg av matvarer ☐
- mindre bevisst i forhold til valg av matvarer ☐
- gjennomført et spesielt kostregime ☐

hvilket……..

- annet……

2. Har du endret kroppsvekt i perioden du har registrert kostholdet?

☐ Ja ☐ Nei

Hvis ja, har du

- gått opp i vekt ☐
- gått ned i vekt ☐

Hvis ja, hvorfor har du endret kroppsvekt?

- bevisst ☐
- sykdom ☐
- treningsbelastning ☐
- annet……

Hvis ja, har dette påvirket kostholdet ditt de dagene du har registrert kostholdet?

- spist mer ☐
- spist mindre ☐
- annet……

3. Har du vært syk i perioden du har registrert kostholdet?

☐ Ja ☐ Nei

Hvis ja, hvordan syk har du vært

- forkjølt/influensa ☐ ant. dager ☐
- feber ☐ ant. dager ☐
- omgangssyke ☐ ant. dager ☐
- mage/tarmproblem ☐ ant. dager ☐
- annet…… ☐ ant. dager ☐

4. Har du brukt medisiner i perioden du har registrert kostholdet?

☐ Ja ☐ Nei

Hvis ja, hvilke

……

Appendix 6: Questionnaire for subject characteristics

ID-nr: Dato:

KARAKTERISTIKA

VEKT (kg)	Nå		
	Før kostregistrering		
	Etter kostregistrering		
HØYDE (cm)			
ALDER (år)			
ETNISITET (land)	Hvor er du født?		
	Hvor er foreldre født?		
RØYKER DU?			
KALSIUM	Melk som barn (0/1-3/4-6/7+)		Glass/d
	Melk nå (0/0,1-1/1,1-3/>3)		Glass/d
	Yoghurt nå		Beger/u
	Ost nå		Brødsk/d
	Iskrem nå		dl/u
FASTE MEDIKAMENTER			

Appendix 7: Questionnaire for supplement use

BRUK AV KOSTTILSKUDD/NATURPREPARAT

1. Bruker du kosttilskudd?

☐ Ja ☐ Nei

Hvis ja, hva bruker du

(Oppgi produktnavnet på det du bruker, hvor ofte og antall tabletter/kapsler/skjeer du tar hver gang)

- Multi-vitamin- og mineralpreparat .....
- Multi-vitaminpreparat .....
- Multi-mineralpreparat .....
- "Pakkeløsninger" .....

Andre vitaminer

- Vit. A .....
- Vit. D .....
- Vit. E .....
- Vit. B .....
- Vit. C .....

Andre mineraler og sporstoff

- Kalsium .....
- Sink .....
- Magnesium .....
- Jern .....
- Andre .....

Andre kosttilskudd

- Tran (flytende, kapsler) .....
- Omega-3 (flytende, kapsler) .....
- Protein .....
- Frie aminosyrer .....
- Kreatin .....
- Karnitin .....
- Naturpreparat/urter.....
- Annet .....

2. Tar du disse preparatene regelmessig

☐ Ja ☐ Nei

Hvis ja, hvor regelmessig

- hele året ☐
- kun i perioder ☐ .....
- faste intervall ☐ .....

Merknader

.....
.....
.....

3. Hvorfor tar du kosttilskudd eller andre naturpreparat?

.....
.....
.....
.....
.....
.....

4. Er det noen som har anbefalt deg å ta kosttilskudd eller andre naturpreparat?

☐ Ja ☐ Nei

Hvis ja, hvem

.....
.....

5. Hvor lenge har du brukt kosttilskudd eller andre naturpreparat?

.....
.....

Appendix 8: Questionnaire for sun exposure and behavior

SPØRRESKJEMA OM SOLVANER

	Dag 1	Dag 2	Dag 3	Dag 4	Dag 5	Dag 6	Dag 7
Timer i dagslys siste 7 dager							

Omtrent hvor mange timer daglig er du ute i dagslys utenom trening om sommeren? ☐

Omtrent hvor mange timer daglig trener du ute om sommeren? ☐

Omtrent hvor mange timer daglig trener du inne om sommeren? ☐

	Ja	Nei
Tilbrakte du hele sommeren (juni-august) 2010 i Norge/Norden?		

	Ja	Nei
Har du vært på solferie i Syden etter september 2010?		

	Ja	Nei
Har du brukt solarium i løpet av den siste måneden?		

	Blir alltid brent	Blir ofte brent	Blir noen ganger litt brent	Blir sjelden brent
Hvordan responderer huden din på akutt soling uten solkrem?				

	Blir aldri brun	Har vanskelig for å bli brun	Blir gradvis brun	Blir lett brun
Hvordan responderer huden din på soling over tid uten solkrem?				

	Alltid (nevn faktor)	Ofte (nevn faktor)	Noen ganger (nevn faktor)	Aldri
Bruker du solkrem?				

Hvilken hårfarge har du?	

Hvilke(n) øyenfarge(r) har du?	

Kommentarer	