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ARTICLE

Staphylococcus aureus nasal carriage is associated with serum 25-hydroxyvitamin D levels, gender and smoking status. The Tromsø Staph and Skin Study

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Abstract Vitamin D induces the expression of antimicrobial peptides with activity against *Staphylococcus aureus*. Thus, we studied the association between serum 25-hydroxyvitamin D (25(OH)D) and *S. aureus* nasal colonization and carriage. Nasal swabs, blood samples and clinical data from 2,115 women and 1,674 men, aged 30–87 years, were collected in the Tromsø Staph and Skin Study 2007–08, as part of the population-based sixth Tromsø Study. Multivariate logistic regression analyses were stratified by recognized risk factors for *S. aureus* carriage: sex, age and smoking. In non-smoking men, we observed a 6.6% and 6.7% decrease in the probability of *S. aureus* colonization and carriage, respectively, by each 5 nmol/l increase in serum 25(OH)D concentration ($P<0.001$ and $P=0.001$), and serum 25(OH)D>59 nmol/l and ≥75 nmol/l as thresholds for ~30% and ~50% reduction in *S. aureus* colonization and carriage. In non-smoking men aged 44–60 years, the odds ratio for *S. aureus* colonization was 0.44 (95% confidence interval, 0.28−0.69) in the top tertile of serum 25(OH)D versus the bottom tertile. In women and smokers there were no such associations. Our study supports that serum vitamin D is a determinant of *S. aureus* colonization and carriage.

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Introduction

The burden of disease from *Staphylococcus aureus* is high and worrying due to widespread antimicrobial resistance [1]. Nasal carriage of *S. aureus* is a major risk factor for infections with the bacterium [2–4]. Since about 20% of healthy adults are persistent nasal carriers [4], prevention or elimination of the carrier state may contribute substantially in reducing the *S. aureus* disease burden. However, there is still limited evidence in relation to modifiable risk factors for the carrier state [5]. Smoking is so far the only protective factor observed across different studies [6, 7], while serum glucose levels [6] and oral contraceptives use [8] have been positively associated with *S. aureus* nasal

carriage. Interestingly, an inverse dose-dependent association was recently observed between serum vitamin D levels and risk of methicillin resistant *S. aureus* (MRSA) nasal carriage, while no association was found for methicillin sensitive *S. aureus* (MSSA) [9].

Vitamin D has direct effects on immunity modulated by the vitamin D receptor (VDR) present in immune cells. Binding of VDR to its responsive element induces expression of antimicrobial peptides (i.e. cathelicidin and β-defensin) with activity against *S. aureus* in vitro [10–12].

Serum 25-hydroxyvitamin D (25(OH)D) provides an overall estimate of vitamin D status and integrates vitamin D derived from endogenous production from sun exposure, and from dietary intake [13]. Importantly, populations living at higher latitudes with periodic lack of photosynthesis show larger proportions of vitamin D insufficiency and increased risk of several chronic and some infectious diseases [13–18]. In the present Norwegian study population, the Tromsø Study, we observed variation in serum 25 (OH)D levels by season, age, body weight, intake of vitamin D, physical activity and smoking [19, 20], and variation in *S. aureus* carriage rates by sex and age [21].

Further studies of the role of vitamin D in the host-microbe interplay may give novel clues to targets for prevention of *S. aureus* carriage and infection as well as underlying biological mechanisms. We therefore examined the cross-sectional relationship between serum 25(OH)D concentration and *S. aureus* nasal colonization and carriage in 4,000 men and women participating in the Tromsø Staph and Skin Study (TSSS), a sub-study of the sixth Tromsø Study, evaluating both possible dose-response and thresholds for adequate immune response against *S. aureus*.

Subjects and methods

Population and study design: The Tromsø Staph and Skin Study (TSSS)

The Tromsø Study is a longitudinal, multipurpose, population-based study in the municipality of Tromsø, Norway, 69°N. In the sixth Tromsø Study (October 2007–December 2008), a total of 12,984 subjects (65.7%) attended [22]. TSSS took place during October 2007 till July 2008 and nasal swab cultures were collected from all attendees aged 30–49 years and random samples of older attendees (relative distribution of birth cohorts as in the municipality). The 4,026 participants who had a first nasal swab culture were invited to a repeated sample within a few weeks (Fig. 1). Participants with missing data on serum 25 (OH)D (n=60) or smoking status (n=48) and those taking antibiotics, either systemic or eye drops/ointments, within 24 h before nasal swabbing (first swab n=27, second swab n=14) were excluded. We included 3,789 participants with minimum one valid nasal swab culture for analysis of *S. aureus* nasal colonization, and 2,780 participants with two valid nasal swab cultures for the analysis of *S. aureus* carriage (Fig. 1).

Information was obtained from questionnaires, interview, clinical examinations and blood samples performed by specially-trained healthcare workers according to standardised procedures. The study was approved by the Regional Committee of Medical and Health Research Ethics, North Norway.

Detection of *S. aureus* nasal colonization and carriage

Both anterior nares were sampled with a NaCl-moistened sterile rayon-tipped swab that was placed in Amies charcoal transport medium (Copan, Brescia, Italy). All specimens were cultured within 3 days at the Department of Microbiology and Infection Control, University Hospital of North Norway (UNN), Tromsø. The swabs were plated on chromID *S. aureus* and chromID™ MRSA agars (bioMérieux, Marcy I'Etoile, France) and incubated for 48 hours at 35°C. Colony morphology on the agar plates was

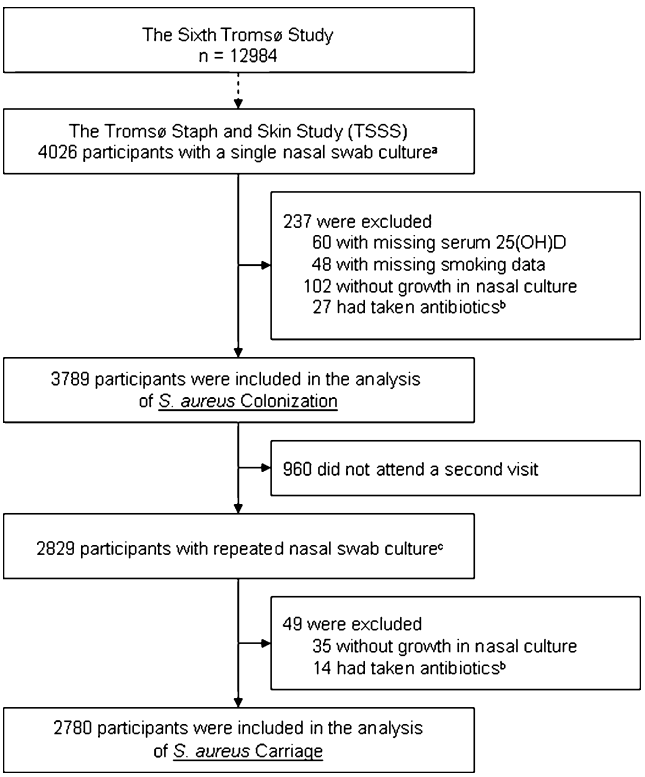

Fig. 1 Study population: *S. aureus* colonization (single nasal swab culture) and *S. aureus* carriage (repeated nasal swab culture). [a]Participants invited; <50 years: all, and ≥50 years: random samples. [b]Antibiotics taken in the last 24 hours before visit: systemic or eye drops/ointments. [c]Median time between repeated nasal swabs was 31 days

the basis for *S. aureus* and MRSA identification. Suspected positive colonies were confirmed as *S. aureus* by the Staphaurex Plus agglutination test (Murex Diagnostic Ltd, Dartford, UK). No MRSA was registered.

S. aureus colonization state was defined as positive or negative for *S. aureus* in the first sample. Carrier state was based on the culturing results of two consecutive samples; carrier = two positive samples and non-carrier = one or no positive sample [23].

Assessment of serum 25-hydroxyvitamin D concentrations

Non-fasting blood samples were drawn from an antecubital vein, and sera were consecutively analysed for 25(OH)D by immunometry (electrochemiluminescence immunoassay), using an automated clinical chemistry analyser (Modular E170; Roche Diagnostics [20, 24]). The total analytical coefficient of variation was 7.3%. We recently showed that smokers had 15–20% higher serum 25(OH)D than non-smokers. However, this was not observed when using other immunological and liquid-chromatography mass spectrometry methods [19]. To this discrepancy, presently, we have no explanation. Thus, non-smokers and smokers are analysed separately.

Assessment of characteristics of the study population

Two self-administered structured questionnaires covered a broad range of issues related to health and lifestyle. Body height and weight were measured, and body mass index was calculated (BMI kg/m^2) [22]. Use of antibiotics in the last 24 hours was registered by interview.

Statistical analysis

Logistic regression models were used to study the association between serum 25(OH)D and *S. aureus* colonization and carriage; odds ratios (ORs) and 95% confidence intervals (CIs) were determined. Due to lack of prior knowledge of serum 25(OH)D thresholds for adequate immune response, serum 25(OH)D tertiles were selected as suitable for the samples; non-smokers were subdivided as: <44.9 nmol/l, 44.9–58.6 nmol/l, >58.6 nmol/l; and smokers: <59.6 nmol/l, 59.6–75.3 nmol/l, >75.3 nmol/l. Also, proposed cut-points for vitamin D deficiency/insufficiency were examined (i.e. < 50.0, 50.0–<75.0, ≥75.0 nmol/l) [14] among non-smokers. Selected characteristics of men and women in the different serum 25(OH)D tertiles were compared by one-way ANOVA and Kruskal-Wallis test for continuous variables and two-sided Pearson chi-squared test for categorical variables.

We evaluated model fit and biological plausibility of several covariates and the final multivariate models included age, BMI, diabetes mellitus (yes/no), and calendar month (2 months categories), and in smokers also number of cigarettes smoked per day and total years smoked. Further adjustment for education (< or ≥ college/university degree), last hospitalization in 12 months (yes/no), recreational physical activity (3 levels), and alcohol intake (< or ≥2 times a week) did not alter the multivariate risk estimates.

We studied whether age modified the association in stratified logistic regression models using tertiles of age for non-smokers grouped as: <44 years, 44–60 years, >60 years.

Tests of reliability of the final analyses were done by the Hosmer-Lemeshow goodness of fit test. Tests for linear trend were performed by assigning consecutive integers to each tertile of serum 25(OH)D, and testing whether the slope coefficient differed from zero using the Wald chi-square test. Test for interaction was done by inclusion of the multiplicative term of the two predictor variables in the model. Two-sided *P* values<0.05 were considered statistically significant. STATA version 11.0 (StataCorp) was used.

Results

The characteristics of the non-smoking and smoking TSSS study population are shown in Tables 1 and 2, respectively.

Non-smoking population

Non-smokers constituted 80.7% (1,351 of 1,674) men and 78.3% (1,655 of 2,115) women. Mean serum 25(OH)D concentration was 53.3 nmol/l in non-smoking men and 52.4 nmol/l in non-smoking women. For both sexes, high serum 25(OH)D was associated with higher age, physical activity level and alcohol intake, and in women, there was a negative association with BMI (all *P*-values<0.05) (Table 1). The prevalence of *S. aureus* nasal colonization and carriage was 37.5% (506 of 1,351) and 34.1% (338 of 992) in men, and 24.4% (403 of 1,655) and 21.3% (264 of 1,239) in women, respectively.

There was an inverse dose-response relationship between serum 25(OH)D and *S. aureus* nasal colonization and carriage in non-smoking men (Fig. 2). The estimated beta coefficient equals a 6.6% and a 6.7% decrease in the probability of *S. aureus* colonization and carriage, respectively, by each 5 nmol/l increase in serum 25(OH)D concentration (*P*<0.001 and *P*=0.001; unadjusted). Furthermore, in the multivariate logistic regression analysis we observed a 35% and 33% reduction in colonization and carriage risk in upper versus bottom tertiles of serum 25 (OH)D in men (OR 0.65, 95%CI 0.49–0.87, *P* for trend 0.004; and OR 0.67, 95%CI 0.48–0.95, *P* for trend 0.03, respectively) (Table 3). Also, those with serum 25(OH)D

Table 1 The Tromsø Staph and Skin Study. Characteristics of non-smoking men and women by tertiles of serum 25(OH)D

Characteristic	Single swab culture							
	Serum 25(OH)D (nmol/l)							
	Men (n=1,351[a])				Women (n=1,655[a])			
	Tertile 1 <44.9 (n=438)	Tertile 2 44.9–58.6 (n=464)	Tertile 3 >58.6 (n=449)	P[b]	Tertile 1 <44.9 (n=566)	Tertile 2 44.9–58.6 (n=537)	Tertile 3 >58.6 (n=552)	P[b]
Age (years)	51.9 (12.8)	54.3 (12.8)	56.3 (12.3)	<0.001	53.7 (14.2)	54.4 (12.9)	56.0 (12.4)	0.003
Ethnicity sami	10 (2.6)	8 (1.8)	6 (1.4)	0.50	9 (1.7)	9 (1.9)	7 (1.4)	0.84
Low education[c]	250 (57.5)	256 (55.7)	257 (57.5)	0.81	317 (57.2)	308 (58.3)	324 (59.3)	0.78
BMI (kg/m^2)	27.5 (4.0)	27.7 (3.5)	27.1 (3.3)	0.05	27.4 (5.4)	26.8 (4.7)	25.9 (4.1)	<0.001
Diabetes mellitus	13 (3.1)	24 (5.3)	4 (0.9)	0.001	26 (4.7)	21 (4.0)	21 (3.9)	0.77
Atopic eczema	30 (7.7)	32 (7.5)	32 (7.8)	0.99	52 (10.2)	42 (9.0)	43 (8.8)	0.72
Hospitalization[d]	44 (10.2)	42 (9.1)	46 (10.4)	0.78	71 (12.6)	53 (10.1)	66 (12.2)	0.38
Low physical activity[e]	91 (21.5)	77 (17.2)	54 (12.7)	0.003	118 (22.7)	77 (15.5)	60 (11.6)	<0.001
High alcohol intake[f]	86 (19.7)	115 (25.1)	126 (28.4)	0.01	71 (12.7)	100 (18.9)	136 (24.9)	<0.001
Characteristic	Repeated swab culture							
	Serum 25(OH)D (nmol/l)							
	Men (n=992[a])				Women (n=1,239[a])			
	Tertile 1 <44.9 (n=329)	Tertile 2 44.9–58.6 (n=346)	Tertile 3 >58.6 (n=317)	P[b]	Tertile 1 <44.9 (n=431)	Tertile 2 44.9–58.6 (n=390)	Tertile 3 >58.6 (n=418)	P[b]
Age (years)	52.3 (12.6)	55.0 (12.7)	56.6 (12.3)	<0.001	53.7 (13.9)	54.7 (12.8)	56.0 (11.9)	0.03
Ethnicity sami	7 (2.3)	6 (1.8)	5 (1.7)	0.83	9 (2.2)	3 (0.8)	7 (1.8)	0.31
Low education[c]	196 (59.9)	193 (56.4)	180 (57.1)	0.63	240 (56.9)	227 (59.4)	250 (60.2)	0.59
BMI (kg/m^2)	27.6 (4.1)	27.7 (3.6)	27.2 (3.4)	0.13	27.4 (5.2)	26.8 (4.7)	26.1 (4.2)	<0.001
Diabetes mellitus	9 (2.8)	21 (6.2)	2 (0.7)	<0.001	22 (5.2)	13 (3.5)	17 (4.2)	0.46
Atopic eczema	21 (7.1)	27 (8.4)	19 (6.5)	0.64	40 (10.1)	30 (8.6)	33 (8.8)	0.74
Hospitalization[d]	32 (9.9)	31 (9.0)	33 (10.5)	0.81	53 (12.4)	35 (9.2)	47 (11.4)	0.34
Low physical activity[e]	67 (21.0)	59 (17.6)	37 (12.3)	0.02	83 (20.7)	53 (14.7)	55 (13.9)	0.02
High alcohol intake[f]	63 (19.3)	88 (25.7)	90 (28.9)	0.02	51 (12.5)	75 (19.5)	108 (26.2)	<0.001

Values are given as means (standard deviation) and numbers (%)

BMI body mass index

[a] Numbers may vary due to missing information

[b] Kruskal Wallis test for continuous variables. Pearson chi-square test for categorical variables

[c] Only education below college/university degree

[d] Hospitalization in last 12 months

[e] Sedentary recreational physical activity like watching TV

[f] Alcohol intake≥2 times per week

concentration≥75 nmol/l versus below 50 nmol/l had almost half the risk of *S. aureus* colonization and carriage (OR 0.54, 95%CI 0.35–0.84, P for trend 0.004; and OR 0.52, 95%CI 0.31–0.90, P for trend 0.02, respectively) (Table 3). As *S. aureus* carriage rate in men is inversely related to age [21], we stratified by age tertiles in the multivariate logistic regression analysis. In the middle-age tertile, age 44–60 years, OR for colonization and carriage in top versus bottom tertile of serum 25(OH)D was 0.44 and 0.51 (95% CI 0.28–0.69, P for trend <0.001; and 95%CI 0.30–0.88, P for trend 0.02, respectively), while in younger and older adult men no association was observed (P for interaction 0.10 and 0.45, respectively) (results not shown in figures or tables).

Table 2 The Tromsø Staph and Skin Study. Characteristics of smoking men and women by tertiles of serum 25(OH)D

Characteristic	Single swab culture							
	Serum 25(OH)D (nmol/l)							
	Men (n=323[a])				Women (n=460[a])			
	Tertile 1 <59.6 (n=115)	Tertile 2 59.6–75.3 (n=122)	Tertile 3 >75.3 (n=86)	P[b]	Tertile 1 <59.6 (n =143)	Tertile 2 59.6–75.3 (n=140)	Tertile 3 >75.3 (n=177)	P[b]
Age (years)	51.1 (12.3)	51.9 (11.5)	53.0 (10.9)	0.35	52.1 (12.4)	53.4 (10.8)	52.1 (10.6)	0.40
BMI (kg/m^2)	26.7 (4.3)	26.8 (3.5)	26.9 (4.3)	0.99	27.0 (5.3)	25.6 (4.5)	24.7 (3.6)	0.001
No. of cigarettes/day	12.8 (9.6)	13.0 (6.6)	13.6 (6.0)	0.07	9.4 (6.9)	10.9 (4.7)	11.1 (5.2)	0.001
No. of years smoked	30.4 (12.8)	32.9 (11.5)	33.9 (11.7)	0.06	28.8 (11.1)	31.7 (10.2)	32.8 (9.8)	0.004
Characteristic	Repeated swab culture							
	Serum 25(OH)D (nmol/l)							
	Men (n=233[a])				Women (n=316[a])			
	Tertile 1 <59.6 (n=82)	Tertile 2 59.6–75.3 (n=88)	Tertile 3 >75.3 (n=63)	P[b]	Tertile 1 <59.6 (n=97)	Tertile 2 59.6–75.3 (n=98)	Tertile 3 >75.3 (n=121)	P[b]
Age (years)	50.8 (12.1)	53.1 (11.3)	54.0 (11.4)	0.12	52.2 (11.9)	53.7 (11.3)	53.2 (10.2)	0.47
BMI (kg/m^2)	27.2 (4.5)	26.9 (3.3)	26.6 (3.8)	0.55	27.5 (5.6)	25.4 (4.2)	24.6 (3.6)	0.001
No. of cigarettes/day	12.7 (10.2)	13.0 (7.0)	12.7 (5.2)	0.22	9.2 (7.0)	10.6 (4.6)	11.5 (5.7)	<0.001
No. of years smoked	30.3 (12.1)	33.6 (11.6)	34.6 (12.1)	0.06	28.7 (11.2)	32.0 (10.3)	33.7 (9.6)	0.002

Values are given as means (standard deviation) and numbers (%)

BMI body mass index

[a] Numbers may vary due to missing information

[b] Anova and Kruskal-Wallis test for continuous variables. Pearson chi-square test for categorical variables

In non-smoking women, there was a pattern of an inverse trend of the linear regression line in the serum 25 (OH)D–*S. aureus* colonization and carriage plots (P=0.25, and P=0.22, respectively; unadjusted) (Fig. 2), but there was no difference in *S. aureus* nasal colonization and carriage risk between tertiles or categories (i.e. cut-off values 50 and 75 nmol/l) of serum 25(OH)D (Table 3).

As general recommendations on vitamin D status in adults do not differ by sex, we examined the total population of non-smokers and observed a 3.8% and 4.4% decrease in *S. aureus* colonization and carriage risk by each 5 nmol/l increase in serum 25(OH)D concentration, respectively (P=0.001 and P=0.002; unadjusted) (Fig. 2).

Smoking population

In the smoking population, average vitamin D concentration was higher than in non-smokers; mean serum 25 (OH)D concentration was 66.8 nmol/l in men and 71.3 nmol/l in women. In women, serum 25(OH)D was positively associated with number of cigarettes smoked per day and years of smoking (both P-values<0.05) (Table 2). The prevalence of *S. aureus* nasal colonization and carriage was 29.1% (94 of 323) and 24.5% (57 of 233) in men, and 18.3% (84 of 460) and 15.2% (48 of 316) in women, respectively. All prevalence rates were significantly lower than in non-smokers (all P-values<0.05). We did not observe any association between serum 25(OH)D concentration and *S. aureus* nasal colonization or carriage rates in top versus bottom tertile of serum 25(OH)D in either male (multivariate model; colonization: OR 1.19; 95%CI 0.62–2.29, P for trend 0.66; and carriage: OR 1.33, 95%CI 0.53–3.33, P for trend 0.47) or female smokers (multivariate model; colonization: OR 0.96, 95% CI 0.50–1.86, P for trend 0.83; and carriage OR 1.49, 95%CI 0.59–3.77, P for trend 0.48) (results not shown in figures or tables).

Discussion

In this large population-based study with repeated nasal swab cultures we observed an inverse dose-response

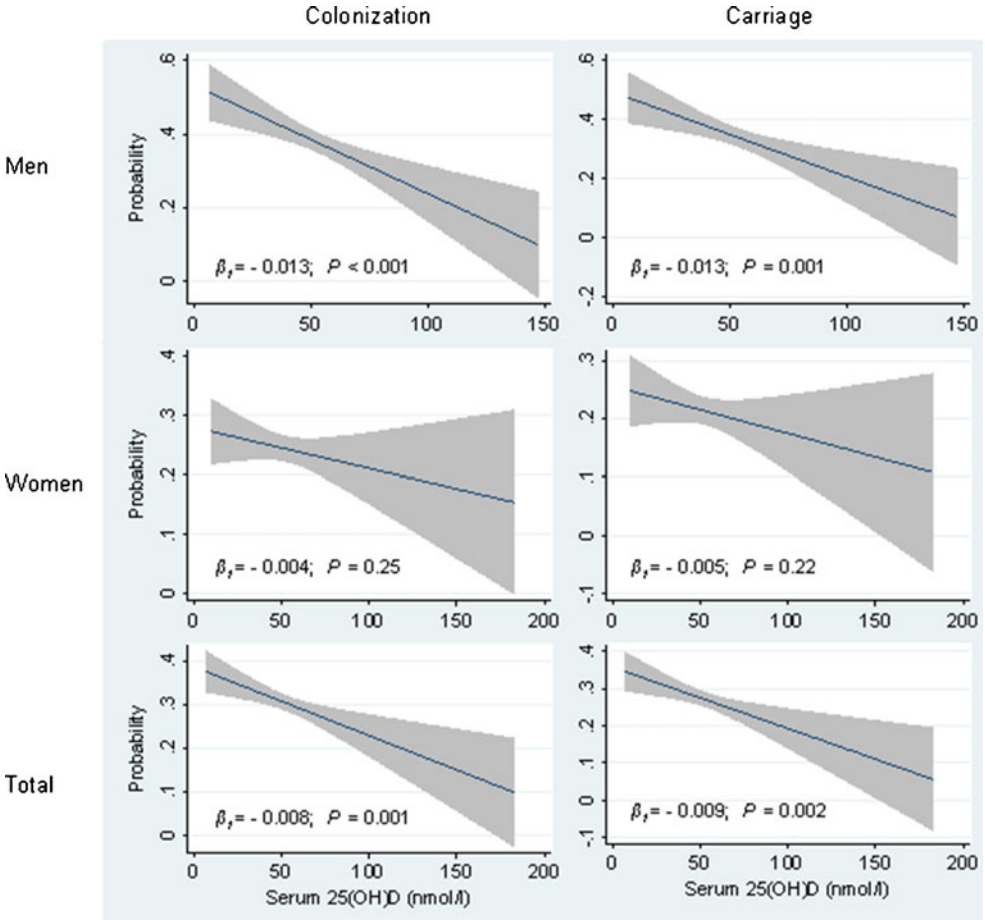

Fig. 2 Probability of *Staphylococcus aureus* colonization and carriage in non-smoking men (n=1,351 and n=992), women (n=1,655 and n=1,239), and total population (n=3,006 and n=2,231), respectively, according to serum 25-hydroxyvitamin D (25(OH)D) level in nmol/l. Lines depict regression line (*navy*) with 95% mean prediction interval (*grey area*)

association between serum 25(OH)D and *S. aureus* nasal colonization and carriage in non-smoking men with no such clear association among women. Based on our findings, we suggest that vitamin D can upregulate the antibacterial immune response and thereby prevent *S. aureus* colonization and carriage, and subsequent disease. Furthermore, we hypothesize that the relative importance of vitamin D in this context is particularly high in the male population who lack female sex hormones known to boost the immune defence [25–27]. *S. aureus* was significantly less frequent in smokers than in non-smokers, and interestingly, serum 25 (OH)D levels did not vary by *S. aureus* colonization or carriage states in smokers. These findings support that the inverse association between vitamin D and *S. aureus* colonization and carriage may be masked by smoking, which is possibly explained by the bactericidal activity of cigarette smoke [7] and the increased immune activity associated with smoking-induced hypoxia.

This study is, to our knowledge, the first to report an association between vitamin D levels and MSSA carriage in a general population. However, in a recent study including single nasal swab cultures from 14,000 children and adults across the USA, an inverse association between vitamin D levels and risk of MRSA was observed [9]. The microbe-dependent association may partly be due to the increased resistance of MRSA to natural antimicrobial peptides (i.e. cathelicidin) induced by vitamin D in host defence against *S. aureus* [28]. The apparent discrepancy with our MSSA results may be explained by several factors. While in Matheson et al. more detailed subgroup analysis was not presented [9], we observed that gender and smoking status may modify the association between vitamin D levels and *S. aureus* colonization and carriage. Furthermore, our study included men and women from a well-defined arctic adult source population, 69°N, and there is minimal concern about geographical and ethnical heterogeneity that, in contrast, may have influenced the findings by Matheson et al. [9].

The inverse dose-response relationship between vitamin D status and *S. aureus* prevalence observed among non-smokers in our study is in accordance with former findings [9], and points to targets for reducing the reservoir of *S. aureus* in the population, in particular when vitamin D insufficiency is common. Carriage of *S. aureus* precedes infection. Thus, our findings suggest that vitamin D supplementation may reduce the incidence of MSSA and

Table 3 The Tromsø Staph and Skin Study. Estimated odds ratios (ORs) for *S. aureus* nasal colonization and carriage in non-smoking men and women by serum 25(OH)D tertiles and categories

Swab culture	Men				Women			
Single swab culture, 25(OH)D tertiles								
25(OH)D (nmol/l)	Total, *n*	Colonized, *n* (%)	OR[a] (95% CI)	OR[b] (95% CI)	Total, *n*	Colonized, *n* (%)	OR[a] (95% CI)	OR[b] (95% CI)
<44.9	438	184 (42.0)	1.0	1.0	566	142 (25.1)	1.0	1.0
44.9–58.6	464	184 (39.7)	0.95 (0.73–1.24)	0.92 (0.70–1.21)	537	130 (24.2)	0.95 (0.73–1.25)	1.00 (0.76–1.32)
>58.6	449	138 (30.7)	0.66 (0.50–0.88)	0.65 (0.49–0.87)	552	131 (23.7)	0.93 (0.71–1.22)	0.99 (0.75–1.31)
p trend			0.004	0.004			0.60	0.94
Repeated swab culture, 25(OH)D tertiles								
25(OH)D (nmol/l)	Total, *n*	Carriers, *n* (%)	OR[a] (95% CI)	OR[b] (95% CI)	Total, *n*	Carriers, *n* (%)	OR[a] (95% CI)	OR[b] (95% CI)
<44.9	329	123 (37.4)	1.0	1.0	431	96 (22.3)	1.0	1.0
44.9–58.6	346	129 (37.3)	1.06 (0.77–1.46)	1.04 (0.75–1.43)	390	83 (21.3)	0.94 (0.67–1.31)	1.02 (0.72–1.43)
>58.6	317	86 (27.1)	0.68 (0.49–0.96)	0.67 (0.48–0.95)	418	85 (20.3)	0.88 (0.63–1.23)	0.95 (0.68–1.33)
p trend			0.03	0.03			0.46	0.76
Single swab culture, 25(OH)D categories								
25(OH)D (nmol/l)	Total, *n*	Colonized, *n* (%)	OR[a] (95% CI)	OR[b] (95% CI)	Total, *n*	Colonized, *n* (%)	OR[a] (95% CI)	OR[b] (95% CI)
<50.0	621	257 (41.4)	1.0	1.0	783	197 (25.2)	1.0	1.0
50.0–<75.0	603	215 (35.7)	0.81 (0.64–1.02)	0.80 (0.63–1.02)	713	171 (24.0)	0.94 (0.74–1.19)	1.0 (0.79–1.28)
>=75.0	127	34 (26.8)	0.57 (0.37–0.88)	0.54 (0.35–0.84)	159	35 (22.0)	0.84 (0.56–1.26)	0.88 (0.58–1.34)
p trend			0.006	0.004			0.38	0.68
Repeated swab culture, 25(OH)D categories								
25(OH)D (nmol/l)	Total, *n*	Carriers, *n* (%)	OR[a] (95% CI)	OR[b] (95% CI)	Total, *n*	Carriers, *n* (%)	OR[a] (95% CI)	OR[b] (95% CI)
<50.0	471	177 (37.6)	1.0	1.0	592	134 (22.6)	1.0	1.0
50.0–<75.0	426	139 (32.6)	0.84 (0.64–1.12)	0.84 (0.63–1.11)	524	108 (20.6)	0.88 (0.66–1.17)	0.92 (0.69–1.23)
>=75.0	95	22 (23.2)	0.55 (0.33–0.92)	0.52 (0.31–0.90)	123	22 (17.9)	0.73 (0.44–1.21)	0.78 (0.47–1.29)
p trend			0.02	0.02			0.18	0.32

n numbers, *CI* confidence interval, *OR* odds ratio

[a] Age-adjusted

[b] Multivariate logistic regression model including: age, diabetes mellitus (yes/no), body mass index (BMI), seasonal month divided in 2 month categories

MRSA infections. Importantly, we identified serum 25(OH)D above 59 nmol/l and 75 nmol/l as thresholds for ~30% and ~50% reduction in *S. aureus* colonization and carriage in non-smoking men. Consensus to define a cut-point for vitamin D insufficiency based on serum 25(OH)D levels is lacking. Various cut-points have been proposed based on population-based reference limits or biological indices as parathyroid hormone, calcium absorption, or bone mineral density, but without reference to immune function and infectious diseases [29, 30]. Given the high risk of *S. aureus* infection in combination with malnutrition in specific patient populations (i.e. surgical, dialysis, ICU, HIV [4]), and the fact that most of the infections are caused by the patient's nasal strain [3, 4], our finding suggests that vitamin D repletion reaching serum 25(OH)D above 60–75 nmol/l may be a significant alternative in the prevention of hospital infections. A recent retrospective study including 52 subjects with *Clostridium difficile* and *S. aureus* infections showed a link between low vitamin D status and adverse outcome [31]. Larger and prospective studies are needed to determine a possible role of vitamin D supplementation and repletion in relation to *S. aureus* colonization, carriage and infection.

The inverse relationship between serum 25(OH)D concentration and *S. aureus* colonization and carriage did not reach statistical significance among women in our study. It has been proposed that women are inherently protected from infections by estrogens, which increase immune function [25, 32]. A variety of immunocompetent cells express estrogen receptors, which mediate the antimicrobial effects, i.e. regulating the expression of caspases and cytokines [25–27]. Thus, we hypothesize that the stable, low lifetime prevalence of *S. aureus* carriage in women is mainly explained by endogenous estrogens that may overwhelm the protective effect of vitamin D. The observed gender difference is in accordance with studies of

other outcomes; type 2 diabetes and insulin resistance have been associated with low vitamin D status in men only [33, 34], but so far these gender differences lack explanation.

Recent studies suggest that higher vitamin D status is protective against upper respiratory tract infections [17, 18, 35], and that seasonal influenza might be linked to the wintertime deficiency of vitamin D [15]. Furthermore, vitamin D deficiency has been associated with increased risk of tuberculosis (TB) [36] and immunomodulatory effects of vitamin D and sunlight in TB therapy continue to be revealed [16]. Importantly, however, a U-shaped association between serum 25(OH)D concentration and risk of active TB was recently observed [37], indicating that vitamin D supplementation may have detrimental effects on the immune function among individuals with normal or high vitamin D status.

Our main findings may be biased by the positive association between age and serum 25(OH)D among non-smoking men; higher consumption of traditional marine food like cod liver and fresh cod liver oil, more frequent extended stays in the south, and lower BMI in the elderly may contribute to this association (results not presented). Also, in male participants, age is inversely related to *S. aureus* nasal carriage [21]. Based on this, we included age as a covariate in our regression analysis and stratified by age group (i.e. tertiles) but observed no interaction. However, in the middle-age tertile with subjects relatively evenly distributed in serum 25(OH)D levels and homogenous *S. aureus* frequencies across the age range, the strength of the vitamin D–*S. aureus* associations increased.

Thus, if there is an association between serum 25(OH)D and risk of *S. aureus* colonization and carriage this could be explained by the immunmodulatory effects of vitamin D. *S. aureus* stimulates the conversion of 25-hydroxyvitamin D (25(OH)D) to the active metabolite 1,25-dihydroxyvitamin D (1,25(OH)2D) [12]. Vitamin D stimulates the production of antimicrobial peptides (i.e. cathelicidin and β-defensin) with activity against *S. aureus* [10], and contributes to the formation of an intact epidermal barrier preventing *S. aureus* invasion (i.e. regulation of keratinocytes) [11]. Interestingly, genetic polymorphisms in VDR in combination with type 1 diabetes has been associated with the risk of *S. aureus* colonization and carriage [38, 39].

Detailed studies have shown that there are two carrier states that differ in the immune response to *S. aureus* and risk of infections; persistent carriers and others [23]. In our study, culturing results of two repeated nasal swabs differed only in a minor proportion of the participants (8%); thus classification by colonization state almost equalled carrier state. Many other similar studies have used only one sample [7, 9]. Furthermore, the high participation rate and uniform use of standard and validated clinical and laboratory procedures increase the external validity of our findings [22, 40].

The cross-sectional study design precludes establishing temporality and thus causality of serum 25(OH)D concentrations and *S. aureus* colonization and carriage. Due to our former studies indicating an overestimation of serum 25 (OH)D levels in smokers by the ECLIA (Roche) test [19], we stratified by smoking status and included smoking data as covariates in the analysis of the smoking population. This strengthens the validity of the linear trend estimates. However, estimation of externally valid cut-off values for serum 25(OH)D in smokers is hindered.

In conclusion, our study indicates an inverse association between serum 25(OH)D concentration and the risk of *S. aureus* nasal colonization and carriage in non-smokers, particularly in men. Prospective randomised trials are needed to assess whether increase in circulating vitamin D concentration can effectively decrease the risk of *S. aureus* carriage and subsequent infection.

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Conflicts of interest All authors had no conflicts of interest.

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