# Determinants and Correlates of Serum Undercarboxylated Osteocalcin

Katharina Nimptsch[a] Susanne Hailer[b] Sabine Rohrmann[a] Kurt Gedrich[c]
Günther Wolfram[d] Jakob Linseisen[a, b]

[a]Division of Cancer Epidemiology, German Cancer Research Center, Heidelberg, [b]Unit of Human Nutrition and Cancer Prevention, [c]Department of Marketing and Consumer Research, and [d]Department of Food and Nutrition, Technical University of Munich, Munich, Germany

**Abstract**

***Background/Aims:*** To assess dietary and nondietary determinants of serum undercarboxylated osteocalcin (ucOC) as a measure of vitamin K status. ***Methods:*** UcOC and total intact osteocalcin (iOC) concentrations were determined by specific ELISA tests in serum samples of 231 male and 320 female participants (18–81 years) of the representative, cross-sectional Bavarian Food Consumption Survey II. Determinants of ucOC were investigated by analysis of variance, Spearman's rank correlation coefficients and logistic regression models. ***Results:*** Mean ucOC serum concentration was 2.46 ng/ml in men and 2.34 ng/ml in women. Corresponding means of the ratio of ucOC to iOC (ucOC/iOC) were 0.28 and 0.29. Concentrations of ucOC and iOC, as well as the ratio of ucOC/iOC, strongly depended on the participant's age. UcOC was influenced by smoking status, sports activity, and the season when blood was collected. Dietary intake of the dominant vitamin K sources, green leafy vegetables and dairy products, as well as the plasma concentration of the carotenoid lutein were inversely associated with serum ucOC values. ***Conclusions:*** In studies using serum ucOC as a measure of vitamin K supply, determinants, especially age, need to be taken into account.

## Introduction

The fat-soluble vitamin K physiologically acts as a cofactor for the posttranslational γ-carboxylation of vitamin K-dependent proteins [1]. The term vitamin K refers to a group of compounds that have a 2-methyl-1,4-naphtoquinone ring in common, but differ in the length and structure of their isoprenoid side chain at the 3 position [2]. Phylloquinone (vitamin $K_1$) and the group of menaquinones (vitamin $K_2$) are the K vitamins that naturally occur in human nutrition. Green leafy vegetables and vegetable fats are major sources of phylloquinone. Menaquinones are synthesized by bacteria and, therefore, occur abundantly in fermented dairy products such as cheese [3, 4].

Osteocalcin (OC) is a vitamin K-dependent protein of the bone and is synthesized by osteoblasts during bone matrix formation [5]. It contains three γ-carboxygluta-

mate residues which are responsible for its specific affinity to the hydroxyapatite (HAP) molecule. Only a small fraction of the produced osteocalcin is not bound to bone and, thus, detectable in serum as a marker of bone formation [6]. In a state of vitamin K deficiency, due to insufficient carboxylation capacity, a fraction of osteocalcin does not undergo the complete carboxylation process, which is referred to as undercarboxylated osteocalcin (ucOC). ucOC concentration in serum has been proposed as a sensitive biomarker of vitamin K status, possibly more informative than prothrombin measurement, which assesses hepatic vitamin K status only, and plasma phylloquinone, which is responsive to recent diet [3, 7, 8]. The responsiveness of the carboxylation status of osteocalcin to vitamin K depletion and repletion has been demonstrated in several trials [9–11]. Elevated concentrations of ucOC are associated with increased hip fracture risk [12–14] and reduced bone mineral density [15, 16].

To date, most studies conducted to investigate ucOC analyzed the biomarker with indirect binding assays, utilizing the different affinity of carboxylated and undercarboxylated OC for HAP or barium sulfate. Recently, a specific enzyme-linked immunosorbent assay (ELISA) for ucOC became available [17], which has been reported to be more sensitive than indirect assays [14].

The association between age and ucOC serum levels has been reported inconsistently. While 1 study reported decreasing ucOC concentrations in men and women with age [7], 2 studies found no age effect in males or females [18, 19], and 2 other studies showed increasing concentrations with age in women [20, 21]. The osteoblastic activity is age dependent, leading to changes in osteocalcin synthesis with age [22]. Increasing ucOC concentrations can be related to either increased production of the carboxylation substrate OC (which leads to higher ucOC concentration in the case of unchanged carboxylation capacity) or reduced carboxylation capacity. Therefore, the ratio of ucOC to total intact osteocalcin (ucOC/iOC) seems to be a more accurate measure of carboxylation status, i.e. vitamin K supply, compared to the absolute ucOC concentration.

Beyond the effect of age, little is known about the determinants and biological correlates of ucOC. Also, studies investigating ucOC are often limited to older women, who have an increased risk of osteoporosis; thus, data on various age groups and male subjects are scarce.

Therefore, the aims of our study were (1) to address the effect of age on ELISA-measured ucOC and iOC concentrations and the ratio of ucOC/iOC in a representative sample of the male and female Bavarian population aged 18–81 years, (2) to identify sociodemographic and lifestyle determinants of ucOC, and (3) to investigate the relationship between dietary intake of significant vitamin K sources as well as plasma nutrients and ucOC.

## Subjects and Methods

*Study Design*

The Bavarian Food Consumption Survey II (BVS II) was conducted between September 2002 and June 2003 as a representative cross-sectional study to investigate dietary and lifestyle habits of the Bavarian population. From the German-speaking population of Bavaria, 1,050 subjects aged 13–81 years were recruited by a 3-stage random route-sampling procedure. At baseline, data on subject characteristics, lifestyle, socioeconomic and health status were collected by means of a computer-aided personal interview. Over the following 2 weeks, dietary intake was assessed with three 24-hour diet recalls by telephone (2 weekdays and 1 weekend day). All adult study subjects (≥18 years) who had completed at least 1 dietary recall (n = 879) were invited to their nearest health office for blood sampling and standardized anthropometric measurements within 6 weeks after recruitment. Blood samples could be obtained from 568 participants for further analysis.

The overall participation rate in the study was 71%. Sixty-five percent of the participants invited for blood sampling provided blood. All study participants gave their written informed consent. The study was approved by the local ethics committee.

*Measurements of ucOC and iOC in Serum, and Tocopherols and Carotenoids in Plasma*

Commercially available ELISA tests based on monoclonal antibodies were applied to analyze ucOC (Glu-OC EIA Kit, Takara Biomedical Group, Otsu, Shia, Japan) and iOC (Metra Osteocalcin EIA Kit, Quidel Corporation, Calif., USA) in serum. iOC corresponds to total osteocalcin (independent of carboxylation status) with the strength of the test set to detect only intact (1–49 peptides) osteocalcin molecules and not N- or C-terminal fragments. Intra-assay coefficients of variation of the ucOC and iOC ELISA were 8.1% and 6.0%, respectively.

Concentrations of α-tocopherol, γ-tocopherol, α-carotene, β-carotene, cryptoxanthin, lutein and lycopene were analyzed in EDTA plasma samples by means of HPLC-UV/VIS according to a previously described method [23]. Coefficients of variation were <2.3%.

*Statistical Analyses*

All statistical analyses were performed by the SAS software package, version 9.1 (SAS Institute, Cary, N.C., USA). Of the 568 study participants who provided blood samples, 7 were excluded because of intake of the vitamin K antagonist marcumar, 6 because of missing data on dietary intake and 4 because of insufficient serum volume. Thus, 551 people were included in the statistical analysis. Subjects with missing information on regular sports activities (n = 46) were assumed to be inactive. Women with missing information on their menopausal status (n = 34) were catego-

**Table 1.** Geometric means of ucOC, iOC and ucOC/iOC

| | n | ucOC, ng/ml | | iOC, ng/ml | | ucOC/iOC | |
|---|---|---|---|---|---|---|---|
| | | mean | 95% CI | mean | 95% CI | mean | 95% CI |
| Men | 231 | 2.50 | 2.31–2.71 | 8.94[a] | 8.57–9.32 | 0.28 | 0.26–0.30 |
| Women | 320 | 2.35 | 2.17–2.55 | 8.17[b] | 7.83–8.52 | 0.29 | 0.27–0.31 |
| Men, age groups | | | | | | | |
| <30 years | 23 | 4.00[a] | 3.22–4.98 | 11.66[a] | 10.68–12.72 | 0.34[a] | 0.28–0.42 |
| 30–39 years | 44 | 3.13[a, b] | 2.59–3.78 | 9.35[b] | 8.67–10.08 | 0.33[a] | 0.28–0.40 |
| 40–49 years | 41 | 2.34[b] | 1.93–2.83 | 8.35[b] | 7.74–9.01 | 0.28[a, b] | 0.23–0.34 |
| 50–59 years | 39 | 2.13[b] | 1.73–2.63 | 7.89[b] | 7.26–8.57 | 0.27[a, b] | 0.22–0.33 |
| 60–69 years | 60 | 1.87[b] | 1.52–2.28 | 8.34[b] | 7.70–9.04 | 0.22[b] | 0.18–0.27 |
| ≥70 years | 24 | 1.85[b] | 1.37–2.51 | 8.66[b] | 7.67–9.78 | 0.21[a, b] | 0.16–0.29 |
| Women, age groups | | | | | | | |
| <30 years | 35 | 2.99[a] | 2.45–3.66 | 8.52[a, b] | 7.55–9.62 | 0.35[a] | 0.29–0.43 |
| 30–39 years | 85 | 2.40[a, b] | 2.07–2.78 | 7.54[a] | 6.89–8.25 | 0.32[a, b] | 0.28–0.37 |
| 40–49 years | 81 | 2.03[b] | 1.74–2.36 | 7.07[a] | 6.45–7.74 | 0.29[a, b] | 0.25–0.33 |
| 50–59 years | 46 | 2.48[b] | 2.04–3.01 | 8.47[b] | 7.53–9.53 | 0.29[a, b] | 0.24–0.35 |
| 60–69 years | 48 | 2.14[b] | 1.80–2.55 | 9.13[b] | 8.21–10.15 | 0.23[b] | 0.20–0.28 |
| ≥70 years | 25 | 2.52[b] | 2.02–3.15 | 10.14[b] | 8.87–11.59 | 0.25[a, b] | 0.20–0.31 |
| Menopausal status | | | | | | | |
| Premenopausal | 196 | 2.35 | 2.13–2.58 | 7.60[a] | 7.17–8.05 | 0.31 | 0.28–0.34 |
| Postmenopausal | 124 | 2.36 | 2.11–2.64 | 9.03[b] | 8.44–9.67 | 0.26 | 0.23–0.29 |

Statistics weighted for deviation from the underlying Bavarian population (sex, age, region).
Geometric means for age groups with different superscript letters were significantly different (Scheffé test, $p < 0.05$).

rized as pre- or postmenopausal according to the median age at menopause (48 years) reported by the postmenopausal female participants. Descriptive results were weighted to correct for deviation of the study group from the distribution of gender, age and region in the underlying Bavarian population.

Because of skewed distributions of ucOC, iOC and the ratio of ucOC to iOC (ucOC/iOC) log transformation was performed and geometric means with 95% confidence intervals (CI) are presented. Geometric means of ucOC, iOC and ucOC/iOC were compared across sex, sex-specific 10-year age groups and menopausal status by analysis of variance performed using SAS PROC GLM. Potential determinants of ucOC and ucOC/iOC were analyzed by sex-specific analysis of variance mutually adjusting for age group, smoking status, alcohol intake, highest school degree, BMI, season of blood donation and sports activity. Scheffé's multiple comparison test was used to test for statistical significance between more than 2 groups.

The associations between dietary intake of food groups relevant for vitamin K intake (green leafy vegetables, vegetable fat, dairy products) or plasma nutrients and the ratio of ucOC/iOC were assessed by partial Spearman's rank correlation coefficients corrected for age and energy intake (food groups) or serum cholesterol (plasma nutrients), respectively.

Additionally, the association between food group intake and ucOC was assessed by means of logistic regression, estimating the odds ratio (OR) of having a ratio ucOC/iOC above 0.44 (75th percentile), i.e. of having a low vitamin K supply. OR and 95% CI were calculated by tertiles of the respective food group intake with the lowest tertile being the reference category. The food group tertiles were entered simultaneously in the crude model. The multivariate model included additional adjustments for age (10-year groups), energy intake (tertiles), sex, smoking status (former, never, current), and the season blood was collected. To evaluate linear trend, median intake values of the food group tertiles were included in the crude or multivariate model as continuous variables.

## Results

Unadjusted geometric means of serum concentrations of ucOC, iOC (ng/ml) and the ratio ucOC/iOC according to sex, 10-year age groups and menopausal status in women are presented in table 1. Mean iOC concentration was 8.94 ng/ml in men, which was significantly higher than in women (8.17 ng/ml). The mean absolute ucOC concentrations in men and women were 2.50 and 2.35 ng/ml, respectively. Expression of ucOC relative to iOC resulted in a ratio of ucOC/iOC slightly below 0.30 in men and women. No significant sex differences were observed in ucOC either expressed in ng/ml or as ucOC/iOC. With increasing age, concentrations of iOC and ucOC signifi-

**Table 2.** Multivariate adjusted geometric means of ucOC, and ucOC/iOC, by gender and selected sociodemographic and life-style factors

| | Men | | | | | Women | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | ucOC, ng/ml | | ucOC/iOC | | | ucOC, ng/ml | | ucOC/iOC | |
| | n | mean | 95% CI | mean | 95% CI | n | mean | 95% CI | mean | 95% CI |
| Total | 231 | 2.39 | 2.18–2.62 | 0.27 | 0.25–0.30 | 320 | 2.20 | 2.00–2.42 | 0.29 | 0.26–0.31 |
| Smoking status | | | | | | | | | | |
| Never | 94 | 2.59[a] | 2.21–3.03 | 0.29[a] | 0.25–0.34 | 193 | 2.26 | 2.01–2.54 | 0.29 | 0.25–0.32 |
| Former | 68 | 2.52[a] | 2.12–3.00 | 0.30[a] | 0.25–0.35 | 60 | 2.32 | 1.94–2.77 | 0.32 | 0.27–0.38 |
| Current | 69 | 1.85[b] | 1.56–2.20 | 0.21[b] | 0.18–0.25 | 67 | 2.06 | 1.73–2.44 | 0.26 | 0.22–0.30 |
| Alcohol intake, g/day | | | | | | | | | | |
| 0 to <5 | 63 | 2.02 | 1.69–2.41 | 0.23 | 0.19–0.27 | 183 | 2.47 | 2.20–2.78 | 0.30 | 0.27–0.34 |
| 5 to <15 | 46 | 2.70 | 2.21–3.31 | 0.30 | 0.24–0.36 | 87 | 2.13 | 1.83–2.48 | 0.29 | 0.25–0.34 |
| ≥15 | 122 | 2.22 | 1.96–2.51 | 0.27 | 0.24–0.31 | 50 | 2.04 | 1.67–2.50 | 0.27 | 0.22–0.33 |
| Highest school degree | | | | | | | | | | |
| Basic (0–9 years) | 127 | 2.34 | 2.08–2.64 | 0.26 | 0.23–0.30 | 144 | 2.11 | 1.85–2.39 | 0.28 | 0.25–0.32 |
| Secondary (10–11 years) | 47 | 2.18 | 1.79–2.65 | 0.25 | 0.21–0.31 | 119 | 2.07 | 1.79–2.38 | 0.27 | 0.24–0.31 |
| Higher (12–13 years) | 57 | 2.37 | 1.97–2.86 | 0.28 | 0.23–0.33 | 57 | 2.47 | 2.05–2.98 | 0.31 | 0.26–0.37 |
| BMI | | | | | | | | | | |
| <25 | 79 | 2.31 | 1.97–2.71 | 0.25 | 0.21–0.29 | 151 | 2.30 | 2.03–2.62 | 0.28 | 0.25–0.32 |
| 25–30 | 104 | 2.34 | 2.03–2.70 | 0.27 | 0.23–0.31 | 108 | 2.20 | 1.91–2.54 | 0.28 | 0.24–0.32 |
| >30 | 48 | 2.24 | 1.81–2.76 | 0.27 | 0.22–0.34 | 61 | 2.12 | 1.76–2.55 | 0.30 | 0.25–0.36 |
| Season of blood donation | | | | | | | | | | |
| Spring (March–May) | 79 | 2.38 | 2.05–2.76 | 0.27 | 0.23–0.31 | 86 | 2.51[a] | 2.15–2.92 | 0.28 | 0.24–0.33 |
| Summer (June–Aug.) | 40 | 2.19 | 1.76–2.73 | 0.26 | 0.21–0.32 | 50 | 1.63[b] | 1.35–1.99 | 0.24 | 0.20–0.29 |
| Fall (Sept.–Nov.) | 56 | 2.12 | 1.78–2.54 | 0.25 | 0.21–0.30 | 114 | 2.25[a] | 1.96–2.58 | 0.31 | 0.27–0.36 |
| Winter (Dec.–Feb.) | 56 | 2.51 | 2.06–3.06 | 0.29 | 0.24–0.35 | 70 | 2.58[a] | 2.14–3.11 | 0.32 | 0.26–0.38 |
| Sports | | | | | | | | | | |
| Yes | 117 | 2.52[a] | 2.18–2.92 | 0.28 | 0.24–0.32 | 153 | 2.34 | 2.08–2.65 | 0.27 | 0.23–0.30 |
| No | 114 | 2.09[b] | 1.83–2.39 | 0.25 | 0.22–0.28 | 167 | 2.08 | 1.82–2.38 | 0.31 | 0.27–0.35 |

Geometric means for determinants with different superscript letters were significantly different (Scheffé test, $p < 0.05$).

cantly decreased in men. This inverse relationship still existed when ucOC was expressed as ucOC/iOC. In women, ucOC concentrations declined from the youngest age group (<30 years) to the fourth decade, and then rose nonsignificantly. However, ucOC, expressed as ucOC/iOC, significantly decreased in women with increasing age. UcOC or the ratio of ucOC/iOC did not differ significantly by menopausal status.

Male current smokers had significantly lower serum ucOC concentrations, as well as a lower ratio of ucOC/iOC, than former smokers and nonsmokers (table 2). The same could be observed among females, but the effect did not reach statistical significance. Alcohol intake, educational level and BMI were not associated with multivariate adjusted ucOC concentration or ratio. Men engaging regularly in sports activities had significantly higher ucOC concentrations (ng/ml) compared with those who did not. In the blood samples obtained during summer and fall, ucOC concentrations were lower compared to those obtained during winter or spring, significantly so for women during summer. Other potential determinants including social class, average sleeping time, and use of hormone replacement therapy were investigated, but turned out to be not associated with ucOC (data not shown).

Consumption of green leafy vegetables, the major food source of vitamin $K_1$ (phylloquinone), was significantly inversely correlated with ucOC/iOC ($r = -0.09$, $p = 0.01$), whereas vegetable fats showed no association ($r = -0.02$, $p = 0.67$). Also, intake of dairy products, an important food source for vitamin $K_2$ (menaquinone), was significantly inversely associated with ucOC/iOC ($r = -0.10$, $p = 0.02$). There was a strong inverse correlation between the lutein plasma concentration and ucOC/iOC ($r =$

−0.18, $p < 0.01$), while plasma γ-tocopherol and β-carotene showed weaker associations ($r = -0.09$, $p = 0.04$ and $r = -0.07$, $p = 0.09$, respectively). For α-tocopherol and other carotenoids, correlation coefficients were lower and not significant (data not shown).

Table 3 gives the results of the logistic regression analysis estimating the OR of having high ucOC (ucOC/iOC ≥0.44, equivalent to the 75th percentile) suggestive of a poor vitamin K supply according to tertiles of dietary intake. Subjects in the upper tertile of green leafy vegetable intake had a significantly reduced OR of having high ucOC. As found in the correlation analysis, vegetable fat intake showed no association with ucOC. The intake of dairy products of more than 90 g per day was associated with a 50% reduced OR (adjusted) of having high values of ucOC, i.e. of having a low vitamin K status. Thus, the inverse association of green leafy vegetable and dairy product intake observed in the correlation analysis was confirmed by multivariate logistic regression.

**Table 3.** OR for ucOC ≥0.44 (75th percentile) according to food group intake

| | Crude OR | 95% CI | Adj. OR[1] | 95% CI |
|---|---|---|---|---|
| Green leafy vegetables, g/day | | | | |
| Tertile 1 (<4.3) | 1 | | 1 | |
| Tertile 2 (4.3–31.3) | 0.79 | 0.50–1.26 | 0.80 | 0.49–1.30 |
| Tertile 3 (>31.3) | 0.58 | 0.35–0.94 | 0.58 | 0.35–0.97 |
| p for trend | 0.03 | | 0.05 | |
| Vegetable fat, g/day | | | | |
| Tertile 1 (<2.9) | 1 | | 1 | |
| Tertile 2 (2.94–9.2) | 0.99 | 0.61–1.60 | 1.00 | 0.61–1.66 |
| Tertile 3 (>9.2) | 1.09 | 0.67–1.76 | 1.15 | 0.69–1.92 |
| p for trend | 0.65 | | 0.54 | |
| Dairy products, g/day | | | | |
| Tertile 1 (<90.1) | 1 | | 1 | |
| Tertile 2 (90.1–203.7) | 0.58 | 0.36–0.94 | 0.50 | 0.30–0.82 |
| Tertile 3 (>203.7) | 0.72 | 0.45–1.15 | 0.55 | 0.33–0.91 |
| p for trend | 0.24 | | 0.04 | |

[1] Adjusted for age, sex, energy intake, smoking status, alcohol intake, sport and season of blood collection.

## Discussion

In the present study investigating the determinants and correlates of serum ucOC as a suggested marker of vitamin K supply, ucOC was strongly dependent on the participant's age. In addition, smoking status, sports activity, the season of blood collection as well as dietary intake of green leafy vegetables and dairy products were found to determine the ucOC concentration. UcOC was also significantly inversely correlated with plasma lutein and γ-tocopherol concentrations.

Serum ucOC concentration and ucOC/iOC did not differ significantly between men and women, whereas iOC concentration was higher in men. In the few available studies investigating ucOC in both men and women, no sex differences were observed for ucOC or ucOC relative to total OC, regardless of the method of analysis [7, 18, 24]. However, none of the 3 studies observed significantly higher iOC concentrations in males compared to females.

Higher iOC concentrations with increasing age in women, as observed in the present study, are related to increased bone turnover rates leading to higher OC synthesis [25]. ucOC/iOC shows that the rise in ucOC concentration (ng/ml) observed in women after the fifth decade is attributable to a higher OC synthesis rather than decreased carboxylation activity. Thus, interpreting changes in absolute ucOC concentration as changes in carboxylation activity is prone to error because of the strong dependency of ucOC concentration on OC synthesis. ucOC/iOC is therefore the superior unit to describe ucOC as a biomarker of vitamin K status.

An inverse association of ucOC with age, similar to our results, was found in a study by Sokoll and Sadowski [7] in which ucOC (ng/ml) was measured by barium sulfate assay in males and females aged 18–85 years. In contrast, a Japanese study using specific immunoassays showed increasing ucOC concentrations and ucOC/iOC with age in women [21]. The increase in serum iOC in women over the age of 50 in our study is consistent with the literature [7, 18, 22, 25, 26]. However, we did not find distinct differences in ucOC when comparing pre- and postmenopausal women as observed in other studies [7, 20, 27, 28].

Due to the lack of comparable results, interpreting the associations between smoking status, sports activities and ucOC concentrations in men is difficult. However, some studies have found associations of physical activity and smoking with bone density, which is likely to affect bone metabolism, including osteoblast activity [29]. Seasonal dependency of ucOC concentrations (in terms of higher concentrations in winter and lower concentrations in summer), as reported here, have also been observed in a French sample of elderly women [12]. The

authors of that study suggest that ucOC is not only an indicator of vitamin K status but also depends on the concentration of 25-hydroxyvitamin D, which has been shown to stimulate γ-carboxylase activity [30]. Therefore, seasonal variations of ucOC may be partly explained by seasonal variations in vitamin D supply related to higher sun-induced endogenous vitamin D synthesis in summer. On the other hand, when considering ucOC concentration as a marker of vitamin K status reflective of vitamin K intake, higher intakes of fresh green leafy vegetables rich in vitamin K in summer, as observed, e.g. in Germany [31], may also contribute to the seasonal variation of ucOC concentrations.

The obtained results concerning the associations between sociodemographic and lifestyle factors and the marker of vitamin K status, ucOC, are generally in agreement with similar analyses investigating determinants of plasma phylloquinone as a marker of vitamin K supply.

The intake of green leafy vegetables, the food group that has been shown to account for 30–50% of daily vitamin K intake in the UK and the US [4, 32, 33], was inversely associated with ucOC. Vegetable fat, as a minor phylloquinone source, turned out not to be associated with ucOC. Although certain vegetable fats contain relatively high amounts of phylloquinone, the absolute intake of vegetable fats in g/day is rather low compared to the intakes of green vegetables or dairy products. Also, the food group 'vegetable fat' only includes added fats and does not account for fats in processed food, e.g. in cakes or sauces. The major food sources of menaquinones, dairy products, were inversely associated with ucOC to a similar extent as green leafy vegetables. Therefore, menaquinone may have a significant influence on vitamin K status, despite contributing less (approx. 20%) to total vitamin K intake than phylloquinone. This could be because of its higher bioavailability and longer half-life time in blood [34].

We analyzed the association between dietary intake and ucOC on the level of food groups instead of the individual nutrient level. This has the advantage that recommendations on how to improve vitamin K status can be directly derived from the analysis results. However, analysis of the association between vitamin K intake and ucOC is of great interest concerning the use of ucOC as a biomarker of vitamin K intake. To date, no food content data are available for Germany regarding vitamin K that are exclusively based on HPLC-measured values, which are considered as the most reliable. Therefore, we decided to analyze food groups, as they are a better indicator of vitamin K intake.

The strong inverse correlation between plasma lutein concentration and ucOC/iOC is likely to be attributable to the coexistence of lutein and phylloquinone in green leafy vegetables on the one hand [35], and similarities in the transport by lipoproteins on the other hand [36]. The main food sources of other fat-soluble plasma analytes, such as γ-tocopherol and β-carotene, are less congruent to those of vitamin K, which is reflected by weaker relationships with ucOC concentration.

In the present study, serum ucOC and iOC concentrations were measured using specific ELISA tests. The ELISA test for ucOC has a higher sensitivity and specificity than the more common HAP assay, in which a significant fraction of ucOC is bound nonspecifically by HAP and, therefore, not analyzed as ucOC [14]. Also, analysis of ucOC by ELISA can be conducted more quickly and requires less volume than the HAP assay, which is an additional advantage of this method. The absolute concentrations of ucOC observed here were comparable to the baseline concentrations in 2 Japanese intervention trials using the same assay [11, 28], while substantially higher concentrations (mean ucOC 6.2 ng/ml) were reported in an Irish study on postmenopausal women [19].

When analyzing a (suggested) biomarker of nutrient intake in a representative population sample, one usually intends to describe the prevalence of optimal supply versus suboptimal supply versus deficiency. To date, there is no generally accepted cut-off value of ucOC to detect a suboptimal vitamin K supply, although several thresholds have been suggested depending on the method of analysis [12, 18, 37]. Thus, we chose the 75th percentile of the ucOC/iOC distribution as a cut-off value which turned out to be appropriate to discriminate between subjects for the purpose of logistic regression analysis. However, more efforts to determine an upper limit of an acceptable ucOC serum concentration or ucOC/iOC would be welcome to support the practical usefulness of ucOC as a marker of vitamin K supply. Such cut-off values need to refer to the method of analysis of ucOC, since it has been shown that, despite good correlations, ucOC concentrations determined by ELISA exceed HAP-measured concentrations by about 30% [14].

This study provides an insight into the determinants of ucOC in a medium-sized representative population sample, and, due to the large number of male subjects, contributes to the limited data available on ucOC in men. The wide age range of the participants made it possible to study the age effects explicitly. Comparison of the serum ucOC concentration (ng/ml) with ucOC/iOC revealed

that the ucOC/iOC ratio is the preferable unit to use when reporting ucOC status as a marker of vitamin K supply. Due to the strong age dependency of ucOC, in studies linking ucOC and disease, a narrow age range or matching by age would be helpful to control the age effect. Appraisal of individual vitamin K status by ucOC concentration or the ratio of ucOC/iOC remains difficult due to the lack of a reliable cut-off level for suboptimal vitamin K supply.


## Acknowledgements

We gratefully acknowledge the cooperation of the study participants as well as the work of all co-workers involved in the sampling of data and biological specimens. We especially thank the physicians from the health offices in Bavaria for providing study rooms and for drawing the blood samples and Prof. Georg Karg for his major contribution to the BVS II study.

The study was supported by funds from the Bavarian Ministry of Health and Consumer Protection, and the Kurt Eberhard Bode Foundation.

Katharina Nimptsch is recipient of a scholarship from the Deutsche Forschungsgemeinschaft, Graduiertenkolleg 793.


## References


1 Furie B, Bouchard BA, Furie BC: Vitamin K-dependent biosynthesis of gamma-carboxyglutamic acid. Blood 1999;93:1798–1808.
2 Shearer MJ: Role of vitamin K and Gla proteins in the pathophysiology of osteoporosis and vascular calcification. Curr Opin Clin Nutr Metab Care 2000;3:433–438.
3 Vermeer C, Shearer MJ, Zittermann A, Bolton-Smith C, Szulc P, Hodges S, et al: Beyond deficiency: potential benefits of increased intakes of vitamin K for bone and vascular health. Eur J Nutr 2004;43:325–335.
4 Booth SL, Suttie JW: Dietary intake and adequacy of vitamin K. J Nutr 1998;128:785–788.
5 Zittermann A: Effects of vitamin K on calcium and bone metabolism. Curr Opin Clin Nutr Metab Care 2001;4:483–487.
6 Weber P: Vitamin K and bone health. Nutrition 2001;17:880–887.
7 Sokoll LJ, Sadowski JA: Comparison of biochemical indexes for assessing vitamin K nutritional status in a healthy adult population. Am J Clin Nutr 1996;63:566–573.
8 Thane CW, Bates CJ, Shearer MJ, Unadkat N, Harrington DJ, Paul AA, et al: Plasma phylloquinone (vitamin $K_1$) concentration and its relationship to intake in a national sample of British elderly people. Br J Nutr 2002;87:615–622.
9 Booth SL, Martini L, Peterson JW, Saltzman E, Dallal GE, Wood RJ: Dietary phylloquinone depletion and repletion in older women. J Nutr 2003;133:2565–2569.
10 Binkley NC, Krueger DC, Engelke JA, Foley AL, Suttie JW: Vitamin K supplementation reduces serum concentrations of under-gamma-carboxylated osteocalcin in healthy young and elderly adults. Am J Clin Nutr 2000;72:1523–1528.
11 Tsukamoto Y, Ichise H, Kakuda H, Yamaguchi M: Intake of fermented soybean (natto) increases circulating vitamin $K_2$ (menaquinone-7) and gamma-carboxylated osteocalcin concentration in normal individuals. J Bone Miner Metab 2000;18:216–222.
12 Szulc P, Chapuy MC, Meunier PJ, Delmas PD: Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture in elderly women. J Clin Invest 1993;91:1769–1774.
13 Szulc P, Chapuy MC, Meunier PJ, Delmas PD: Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture: a three year follow-up study. Bone 1996;18:487–488.
14 Vergnaud P, Garnero P, Meunier PJ, Breart G, Kamihagi K, Delmas PD: Undercarboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women: the EPIDOS Study. J Clin Endocrinol Metab 1997;82:719–724.
15 Booth SL, Broe KE, Peterson JW, Cheng DM, Dawson-Hughes B, Gundberg CM, et al: Associations between vitamin K biochemical measures and bone mineral density in men and women. J Clin Endocrinol Metab 2004;89:4904–4909.
16 Szulc P, Arlot M, Chapuy MC, Duboeuf F, Meunier PJ, Delmas PD: Serum undercarboxylated osteocalcin correlates with hip bone mineral density in elderly women. J Bone Miner Res 1994;9:1591–1595.
17 Lee AJ, Hodges S, Eastell R: Measurement of osteocalcin. Ann Clin Biochem 2000;37:432–446.
18 McKeown NM, Jacques PF, Gundberg CM, Peterson JW, Tucker KL, Kiel DP, et al: Dietary and nondietary determinants of vitamin K biochemical measures in men and women. J Nutr 2002;132:1329–1334.
19 Collins A, Cashman KD, Kiely M: Phylloquinone (vitamin $K_1$) intakes and serum undercarboxylated osteocalcin levels in Irish postmenopausal women. Br J Nutr 2006;95:982–988.
20 Beavan SR, Prentice A, Stirling DM, Dibba B, Yan L, Harrington DJ, et al: Ethnic differences in osteocalcin gamma-carboxylation, plasma phylloquinone (vitamin $K_1$) and apolipoprotein E genotype. Eur J Clin Nutr 2005;59:72–81.
21 Tsugawa N, Shiraki M, Suhara Y, Kamao M, Tanaka K, Okano T: Vitamin K status of healthy Japanese women: age-related vitamin K requirement for gamma-carboxylation of osteocalcin. Am J Clin Nutr 2006;83:380–386.
22 Delmas PD, Stenner D, Wahner HW, Mann KG, Riggs BL: Increase in serum bone gamma-carboxyglutamic acid protein with aging in women. Implications for the mechanism of age-related bone loss. J Clin Invest 1983;71:1316–1321.
23 Hess D, Keller HE, Oberlin B, Bonfanti R, Schuep W: Simultaneous determination of retinol, tocopherols, carotenes and lycopene in plasma by means of high-performance liquid chromatography on reversed phase. Int J Vitam Nutr Res 1991;61:232–238.
24 Duggan P, O'Brien M, Kiely M, McCarthy J, Shanahan F, Cashman KD: Vitamin K status in patients with Crohn's disease and relationship to bone turnover. Am J Gastroenterol 2004;99:2178–2185.
25 Eastell R, Delmas PD, Hodgson SF, Eriksen EF, Mann KG, Riggs BL: Bone formation rate in older normal women: concurrent assessment with bone histomorphometry, calcium kinetics, and biochemical markers. J Clin Endocrinol Metab 1988;67:741–748.
26 Epstein S, Poser J, McClintock R, Johnston CC Jr, Bryce G, Hui S: Differences in serum bone GLA protein with age and sex. Lancet 1984;1:307–310.
27 Plantalech L, Guillaumont M, Vergnaud P, Leclercq M, Delmas PD: Impairment of gamma carboxylation of circulating osteocalcin (bone gla protein) in elderly women. J Bone Miner Res 1991;6:1211–1216.
28 Takahashi M, Naitou K, Ohishi T, Kushida K, Miura M: Effect of vitamin K and/or D on undercarboxylated and intact osteocalcin in osteoporotic patients with vertebral or hip fractures. Clin Endocrinol (Oxf) 2001;54:219–224.

29 Nguyen TV, Kelly PJ, Sambrook PN, Gilbert C, Pocock NA, Eisman JA: Lifestyle factors and bone density in the elderly: implications for osteoporosis prevention. J Bone Miner Res 1994;9:1339–1346.
30 Karl PI, Friedman PA: Effects of parathyroid hormone and vitamin D on the renal vitamin K-dependent carboxylating system. J Biol Chem 1983;258:12783–12786.
31 Adolf T, Schneider R, Eberhardt W, Hartmann S, Herwig A, Heseker H, et al: Ergebnisse der Nationalen Verzehrsstudie (1985–1988) über die Lebensmittel- und Nährstoffaufnahme in der Bundesrepublik Deutschland. Giessen, Wissenschaftlicher Fachverlag Dr. Fleck, 1995.
32 Fenton ST, Price RJ, Bolton-Smith C, Harrington DJ, Shearer MJ: Nutrient sources of phylloquinone (vitamin $K_1$) in Scottish men and women (abstract). Proc Nutr Soc 1997; 56:301A.
33 Booth SL, Pennington JA, Sadowski JA: Food sources and dietary intakes of vitamin K-1 (phylloquinone) in the American diet: data from the FDA Total Diet Study. J Am Diet Assoc 1996;96:149–154.
34 Schurgers LJ, Vermeer C: Determination of phylloquinone and menaquinones in food. Effect of food matrix on circulating vitamin K concentrations. Haemostasis 2000;30: 298–307.
35 Sommerburg O, Keunen JE, Bird AC, van Kuijk FJ: Fruits and vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes. Br J Ophthalmol 1998;82:907–910.
36 Rock CL, Thornquist MD, Kristal AR, Patterson RE, Cooper DA, Neuhouser ML, et al: Demographic, dietary and lifestyle factors differentially explain variability in serum carotenoids and fat-soluble vitamins: baseline results from the sentinel site of the Olestra Post-Marketing Surveillance Study. J Nutr 1999;129:855–864.
37 Kaneki M, Hosoi T, Ouchi Y, Orimo H: Pleiotropic actions of vitamin K: protector of bone health and beyond? Nutrition 2006;22: 845–852.