CLINICAL STUDY

Retinol, retinol-binding protein 4, abdominal fat mass, peak bone mineral density, and markers of bone metabolism in men: the Northern Osteoporosis and Obesity (NO2) Study

Magnus Högström¹, Anna Nordström¹-² and Peter Nordström¹-³

¹Sports Medicine, Department of Surgical and Perioperative Science, Umeå University, S-901 85 Umeå, Sweden, ²Department of Community Medicine and Rehabilitation, Rehabilitation Medicine, Umeå University, S-901 85 Umeå, Sweden and ³Geriatric Medicine, Department of Community Medicine and Rehabilitation, Umeå University, S-901 85 Umeå, Sweden

(Correspondence should be addressed to P Nordström who is now at Department of Community Medicine and Rehabilitation, Geriatric Medicine, Umeå University, S-901 85 Umeå, Sweden; Email: peter.nordstrom@idrott.umu.se)

Abstract

Context: The association between retinol and bone mineral density (BMD) in males after puberty has not been fully investigated previously.

Objective: To investigate the association between retinol, retinol-binding protein-4 (RBP-4), BMD (g/cm²), abdominal fat mass, and markers of bone metabolism in young men.

Design: Longitudinal study.

Participants: Seventy-eight healthy males with a mean age of 22.6 ± 0.7 years at baseline. A follow-up was conducted in 73 of the participants 2.0 ± 0.4 years later.

Main outcome measures: Associations between serum concentrations of retinol and RBP-4, and BMD of the total body, lumbar spine, and hip, serum concentrations of osteocalcin, and carboxy terminal telopeptide of type 1 collagen (CTX), were investigated.

Results: Both retinol and RBP-4 showed an inverse relationship with that of osteocalcin (r = -0.23 to -0.25, P < 0.05). Levels of RBP-4 (r = 0.26, P = 0.02) and osteocalcin (r = -0.23, P = 0.04) were also related to abdominal fat mass, and the relationship between RBP-4, retinol, and osteocalcin disappeared after adjusting for this influence of abdominal fat mass. Neither retinol nor RBP-4 concentrations were associated with BMD at any site, CTX as baseline, or changes in BMD during the 2-year follow-up period. Levels of RBP-4 showed a strong association with levels of retinol (r = 0.61, P < 0.001).

Conclusion: We found a negative association between the bone formation marker osteocalcin with retinol and RBP-4. The association disappeared when adjusting for the influence of abdominal fat mass. Neither retinol nor RBP-4 were associated with peak BMD in young men.

European Journal of Endocrinology 158 765–770

Introduction

Research on the relationship between vitamin A and bone health has yielded equivocal results. Several groups of investigators have shown that a high dietary intake of vitamin A or high serum concentration of retinol may increase the risk of fracture in both men and women (1–4). Studies have also reported an association between high intake of vitamin A and reduced bone mineral density (BMD) (2, 5, 6). However, several studies have also failed to establish a relationship between serum concentrations of retinol, vitamin A supplementation, and bone density (7–11).

In the circulation, retinol-binding protein 4 (RBP-4) is the transport protein for retinol. This protein is secreted by adipocytes and the liver, and has recently been implicated as a key factor in insulin resistance and obesity (12). Whether RBP-4 is related to BMD is not known.

Peak BMD is achieved in the late second or third decade of life (13, 14). Since attaining a high peak BMD may be of great importance for future bone quality (15), optimizing nutritional factors that influence bone accrual is important. In addition, dietary behavior studies suggest that adolescent eating habits are, to a large extent, maintained into adulthood (16–18). Thus, elevated vitamin A intake early in life may be maintained and influence long-term bone health.

The aim of the present study was to investigate the relationships between serum concentrations of retinol and RBP-4, and bone mass, markers of bone metabolism, and fat mass distribution in a cohort of young males near the time of peak BMD.
Subjects and methods

The study was approved by the Ethics Committee of the Medical Faculty at Umeå University, Umeå, Sweden. Each participant provided informed consent. All data were collected at the Sports Medicine Unit at the Umeå University Hospital.

Subjects

Eighty-four healthy Caucasian males from schools and athletic clubs in Umeå, Sweden were recruited for this longitudinal study, called the Northern Osteoporosis and Obesity Study (NO2 Study). Blood samples were obtained at baseline. One recruit was excluded due to depression and anorexia nervosa, and five recruits failed to provide blood samples, leaving 78 participants. None of the participants had any disease and none were taking any medication known to affect bone metabolism. Of the participants, 73 were available for follow-up examination after a mean of 2 years.

Procedures

Lifestyle questionnaire Physical activity was measured with a questionnaire and defined as the self-reported mean activity associated with sweating or breathlessness during each week of the previous year. Physical activity primarily consisted of ice hockey, soccer, floor ball, distance running, and weight training. The questionnaire was used at baseline and at follow-up and included questions about smoking habits, known illnesses, and medication.

Anthropometry Weight and height were measured while the participants wore light clothing. Weight was measured to the nearest 0.1 kg with a digital scale, and height was measured to the nearest 0.5 cm with a wall-mounted stadiometer.

BMD and abdominal fat mass measures Total body, right hip, and lumbar spine (L2–L4) BMD (g/cm²) were measured using a Lunar DPX-L (Lunar Co., Waukesha, WI, USA) dual-energy X-ray absorptiometer with version 4.6e software. Abdominal fat mass was determined from the total body scan using the region of interest option. The upper part of this region was determined by the processus xiphoideus and the lower part by the coccyx. Lateral, this region was determined by the shoulder joint. Scaling was set to 200 to maximize precision. The coefficient of variation (CV; i.e., S.D./mean) was determined by scanning one person seven times on the same day, with repositioning between each scan. Accordingly, CV values were 0.7% for the total body BMD, ~1% for the total hip and lumbar spine, and 2% for abdominal fat mass. The equipment was calibrated each day using a standardized phantom to detect drifts in the measurements. All scans were made on the same Lunar DPX-L.

Determination of retinol and RBP-4 concentration Serum was obtained under a non-fasting condition from 78 men at baseline (i.e., at 22 years of age), and stored in a dark freezer at −80 °C until analyzed. Human plasma (80 μl) was diluted with 300 μl 2-propanol containing the internal standard, retinol acetate, and the antioxidant, butylhydroxytoluene. After thorough mixing (15 min) and centrifugation (10 min, 4000 g at 10 °C), an aliquot of 7 μl was injected from the supernatant into the HPLC system that was performed with an HP 1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a HP 1100 single wavelength u.v. detector operated at 325 nm. Retinol was separated from the matrix and internal standard on a 4.6 × 25 mm reversed-phase column. A two-point calibration curve was made from plasma calibrators with known retinol concentrations. The column temperature was 40 °C. Recovery was >95%, the method was linear from 0.1 to ≥10 μM, and the limit of detection was 0.01 μM. The relative S.D. was 4.9 (1.2 μM) and 5.8% (1.7 μM). RBP-4 plasma concentration was determined with a radial immunodiffusion technique (Human RBP-4 Nano RID Kit, Binding Site Ltd, Birmingham, UK). The CV value for the method was 10.2% (3.1 μM). This method measures total RBP-4, that is, apo-RBP, free holo-RBP, apo-RBP-TTR, and holo-RBP-TTR.

Measurement of bone metabolism markers Osteocalcin and carboxy terminal telopeptide of type 1 collagen (CTX, Beta-CrossLaps) were measured using direct chemoluminescence, based on the sandwich technique, in which the patient sample and specific antibodies form a complex during the first incubation. During a second incubation, streptavidin-marked microparticles were added, and the substance was then magnetically separated. The chemoluminescence was measured by a photo multiplier, and the CV was 1.7–5.2%, depending on the concentration of the molecule measured.

Statistical analyses

Statistical analyses were conducted using SPSS (version 14.0; SPSS Inc., Chicago, IL, USA) All data are presented as means and S.D. bivariate correlations were measured using Pearson’s correlation coefficient (r). Multiple linear regression was used to model the relationship between retinol and BMD. To allow for a U-shaped relation, a second-degree polynomial was used. The variables used in the different analysis were tested for normal distribution using a Q–Q plot. P < 0.05 was considered statistically significant.
Results

Mean participant age, body constitution, physical activity, and BMD of the total body, spine, and hip at baseline and changes at follow-up, as well as baseline concentration of retinol, RBP-4, osteocalcin, and CTX are presented in Table 1. Bivariate correlations between BMD at different sites, abdominal fat mass, and baseline levels of retinol, RBP-4, and markers of bone metabolism are presented in Table 2. Abdominal fat mass showed a bivariate significant correlation with RBP-4 and osteocalcin. There was also a strong tendency toward a significant correlation between abdominal fat mass and retinol (\(P=0.05\)). Retinol and RBP-4 did not have any significant bivariate relationships with BMD at any site measured at 23 years of age or to changes in BMD between 23 and 25 years of age (data not shown), but showed significant negative associations with osteocalcin (\(r=-0.23, P<0.05\); and \(r=-0.25, P<0.05\); data not shown). These correlations disappeared when adjusting for the influence of abdominal fat mass (\(\beta=-0.18 \text{ to } -0.21, P>0.05\)). RBP-4 also showed a strong association with retinol (\(r=0.61, P<0.001\), data not shown). Levels of vitamin D\(_3\), presented in a previous paper (19) were not related to levels of retinol (\(r=-0.12, P>0.05\)), RBP-4 (\(r=-0.05, P>0.05\)), abdominal fat mass (\(r=-0.20, P>0.05\)), or weight (\(r=0.02, P>0.05\)).

The independent relationship at 23 years of age between retinol, RBP-4, and BMD at the different bone sites were investigated after adjusting BMD for the influence of weight, height, physical activity, and age using linear regression (Table 3). In summary, weight, height, and physical activity were independent significant predictors of BMD at all sites whereas retinol and RBP-4 concentrations were not related to BMD at any sites. As indicated by the adjusted \(R^2\), the fits were close to 30% for all models, indicating that the variation in the included predictors explained ~30% of the variation in BMD between subjects.

Regression models were also fit to investigate whether a linear or U-shaped relationship between retinol as a predictor variable and spine BMD as an outcome variable existed. No evidence was found for either relationship (linear model \(P=0.16\); U-shaped model \(r^2_{\text{retinol}}=0.62, r^2_{\text{retinol}}=0.78\)).

Discussion

In the present study, we show, for the first time, a negative association between retinol, RBP-4, and osteocalcin. Osteocalcin is a bone-specific protein secreted by osteoblasts. The protein is considered to be an indicator of osteoblast activity and is regarded as a marker of bone formation (20–22). Previous experimental studies have found that vitamin A hypervitaminosis decreases bone formation in rats (23), and that the retinol metabolite, retinoic acid, decreases production of alkaline phosphatase, osteocalcin, and interleukin-6 in human osteoblast cells, and simultaneously decreases differentiation of those cells (24). Thus, our results may indicate that retinol decreases bone formation in young men. No previous studies have investigated the relationship between RBP-4 and osteocalcin. The relationship found in our study may have several explanations. As expected, there was a strong relationship between RBP-4 and retinol. Thus, the relationship between RBP-4 and osteocalcin may be influenced by the association found in the present study between retinol and osteocalcin. There is also another possible explanation for the relationship between RBP-4 and osteocalcin. RBP-4 is suggested to be a key factor in insulin resistance and the development of diabetes (12). As this protein is produced by adipocytes, the levels decrease as body weight decreases (12).

Table 1 Age, anthropometric data, abdominal fat mass, physical activity, and bone mineral density at baseline and changes to follow-up, and baseline measures of retinol, RBP-4, CTX, and osteocalcin.

| & Baseline & Mean ± s.d. & Range | Follow-up & Mean ± s.d. & Range |
|---|---|---|---|---|---|---|
| Age (years) | 22.6 ± 0.7 | 20.9–24.6 | 2.0 ± 0.4 | 1.31–2.93 |
| Weight (kg) | 81.7 ± 11.1 | 63.0–117.0 | 1.3 ± 4.3 | –13–8 |
| Height (cm) | 181 ± 6 | 169–197 | 0 ± 1 | –2–1 |
| Abdominal fat mass (kg) | 6.0 ± 2.0 | 2.1–11.2 | 0.5 ± 1.2 | –2.9–2.6 |
| Physical activity (h/week) | 4.7 ± 3.9 | 0–16.0 | –1.1 ± 3.0 | –13–6 |
| Bone mineral density (g/cm\(^2\)) | & Total body & 1.31 ± 0.08 | 1.10–1.48 | 0.00 ± 0.03 | –0.06–0.12 |
| & Hip & 1.26 ± 0.15 | 0.88–1.60 | –0.04 ± 0.05 | –0.18–0.06 |
| & Lumbar spine & 1.31 ± 0.12 | 1.03–1.60 | 0.00 ± 0.05 | –0.11–0.10 |
| Retinol (µM) | 2.43 ± 0.49 | 1.4–4.4 | & Total body & 74.0 ± 19.6 | 36.0–139 |
| & Hip & 325.1 ± 182.1 | 83.9–803.4 | | 30.3 ± 10.1 | 12.9–56.8 |
| PTH (pmol/l) | 1.9 ± 0.9 | 0.5–5.3 | | & Total body & 2.63 ± 0.13 | 2.0–3.2 |

Means, s.d., and ranges are presented. RBP, retinol-binding protein; CTX, carboxy terminal telopeptide of type 1 collagen; PTH, parathyroid hormone.
Quite recently, it was demonstrated that osteocalcin, a protein solely produced by osteoblasts, increases the sensitivity to insulin and decreases fatness (25). In the present material, both levels of RBP-4 and osteocalcin were related to abdominal fat mass. The relationship between RBP-4, retinol, and osteocalcin disappeared when adjusting for the influence of abdominal fat mass. Thus, from the results of the present study, we can draw no certain conclusion about the relationship found between RBP-4, retinol, and osteocalcin.

We also measured serum concentration of CTX in our cohort. CTX is produced during the breakdown of type I collagen, and its serum concentration corresponds to bone resorption (26, 27). In the present study, there were no significant associations between retinol, RBP-4, and CTX, suggesting that increased bone resorption was not related to higher RBP-4 and retinol. Previous studies have suggested that bone resorption and bone formation are coupled in the majority of cases (28, 29). In our study, we also demonstrated a positive relationship between CTX and osteocalcin.

In the present cohort of young Scandinavian men, retinol serum concentration was high (2.43 μM) compared with previously published reference values (1.6–2.1 μM) for comparable populations (30–33). However, in one study, the mean retinol concentration of middle-aged Scandinavian men was similar to that of participants in the present study (3). It is of interest that only five participants used vitamin supplements in low doses, and thus the high concentration of retinol resulted from dietary habits. The relatively high retinol concentration may be related to the fact that in Sweden, some other Nordic countries, and in the United States, milk products are fortified with vitamin A. It has been suggested that retinol concentrations below toxic levels could be harmful to bone health and result in an increased fracture rate (3, 4), and that even moderately elevated vitamin A intake may, over time, contribute to the pathogenesis of osteoporosis (34, 35). Despite the fact that the men in our cohort had high concentrations of retinol, and levels of retinol showed a negative association with levels of osteocalcin, we found no association between retinol and BMD at any site. We could only speculate whether bone health may be influenced if their dietary habits continue as they age, with continuously elevated retinol levels, perhaps over decades.

Recent findings suggest that there is an inverse U-shaped relation between retinol concentration and risk of hip fracture, such that both low and high retinol concentrations may be harmful (4), leaving a narrow window of optimal concentration. This is supported by the Rancho Bernardo Study, in which low and high retinol intakes were associated with reduced BMD (6). We did not, however, observe any evidence of a U-shaped relationship in the present study. This was not surprising, given the high concentrations of vitamin A. Only seven subjects had retinol concentrations below 2.0 μM.

In a recent study (19), we found a strong relationship between levels of vitamin D and BMD in the same cohort as in the present study. These results are of interest also with respect to the present study; since vitamin A has been suggested to antagonize vitamin D’s positive influence on bone mineralization and calcium uptake (23, 36–38). We therefore also investigated whether levels of vitamin A and RBP-4 were associated with levels of vitamin D presented in a previous study (19). No such relationship was found. Given that milk products are fortified with vitamin A in Sweden, and that these products are also a source of vitamin D, such a relationship might be difficult to find.

Table 2: Bivariate correlations of bone mineral density, abdominal fat mass, and serum concentration of osteocalcin, CTX, and parathyroid hormone with retinol and retinol-binding protein in 78 young males.

<table>
<thead>
<tr>
<th>Bone mineral density (g/cm²)</th>
<th>Osteocalcin</th>
<th>CTX</th>
<th>PTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total body</td>
<td>Retinol</td>
<td>RBP-4</td>
<td></td>
</tr>
<tr>
<td>Hip</td>
<td></td>
<td>0.02</td>
<td>−0.10</td>
</tr>
<tr>
<td>Spine</td>
<td>−0.02</td>
<td>0.00</td>
<td>0.12</td>
</tr>
<tr>
<td>Abdominal fat mass</td>
<td>−0.22</td>
<td>0.26</td>
<td>−0.23</td>
</tr>
</tbody>
</table>

*P<0.05; CTX, carboxy terminal telopeptide of type 1 collagen; RBP-4, retinol binding protein 4; PTH, parathyroid hormone.

Table 3: Independent contributions of retinol (a), RBP-4 (b), weight, height, physical activity, and age to BMD in 78 young males. β values, adjusted R² values, and P values are presented.

### a

<table>
<thead>
<tr>
<th>β values</th>
<th>BMD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total body</td>
</tr>
<tr>
<td>Retinol (μM)</td>
<td>−0.09</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.33†</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>−0.29†</td>
</tr>
<tr>
<td>Physical activity (h/week)</td>
<td>0.31†</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.15</td>
</tr>
<tr>
<td>Model fit</td>
<td>Adjusted R²</td>
</tr>
</tbody>
</table>

### b

<table>
<thead>
<tr>
<th>β values</th>
<th>BMD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total body</td>
</tr>
<tr>
<td>RBP-4 (mg/l)</td>
<td>−0.16</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.36†</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>−0.29†</td>
</tr>
<tr>
<td>Physical activity (h/week)</td>
<td>0.27†</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.15</td>
</tr>
<tr>
<td>Model fit</td>
<td>Adjusted R²</td>
</tr>
</tbody>
</table>

*P<0.001, †P<0.01, ‡P<0.05; RPB-4, retinol-binding protein 4; BMD, bone mineral density.
Strengths of the present study include its prospective design and previously characterized cohort of young men. To our knowledge, this is the first study to investigate the relationship of retinol and RBP-4 concentrations with measures of bone formation, bone resorption, and BMD in a cohort of young adults. Limitations of our study are the rather small cohort, where the participants were mainly former athletes, and that vitamin A and carotenoid intake were not examined by food questionnaires. The method used to measure both free and bound RBP-4 is less accurate than the ELISA kit now commercially available, and less accurate than the method used to measure total retinol. The markers of bone metabolism could be affected by food intake and day-to-day variations, since these samples were not obtained at the same fasting time every day. A larger measurement error would, however, bias the correlation coefficients for RBP-4 and levels of osteocalcin toward zero.

In conclusion, we observed a negative association between retinol, RBP-4, and osteocalcin in young men. However, this association disappeared when adjusting for the influence of abdominal fat mass. In this rather small cohort, we found no evidence for an influence of retinol on peak BMD in men.

Acknowledgements
The present study was supported by grants from the Swedish National Center for Research in Sports (project number 112/01).

References


29 Parfitt AM. The coupling of bone formation to bone resorption: a critical analysis of the concept and of its relevance to the pathogenesis of osteoporosis. Metabolic Bone Disease and Related Research 1982 4 1–6.


Received 27 December 2007
Accepted 30 January 2008