DECREASED RETINOL TRANSPORT PROTEINS IN THAI POST-MENOPAUSAL WOMEN WITH OSTEOPOROSIS

Chaowanee Chupeerach[1], Talabporn Harnroongroj[1], Benjaluck Phonrat [2], Anchalee Tungtrongchitr [3], Florian J Schweigert[4], Rungsunn Tungtrongchitr[1] and Sangchai Preutthipan[5]

[1]Department of Tropical Nutrition and Food Science, [2]Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok; [3]Department of Parasitology, Faculty of Medicine at Siriraj Hospital, Mahidol University, Bangkok; [5]Department of Obstetrics and Gynecology, Faculty of Medicine at Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [4]Institute of Nutritional Science, Department of Physiology and Pathophysiology of Nutrition, University of Potsdam, Germany

Abstract. High vitamin A ingestion or high serum retinol have been postulated to increase the risk of fractures and osteoporosis by reduced bone mineral density (BMD). Retinol is carried and transported to the tissues bound to retinol binding protein 4 (RBP4) and transthyretin (TTR). The relationships between retinol, retinol transport protein, retinol binding protein 4 (RBP4) and transthyretin (TTR) and BMD and osteoporosis are unclear. To examine the association between retinol and RBP4 and TTR and osteoporosis, 73 osteoporotic and 71 normal Thai post-menopausal women were studied. RBP4 and retinol levels did not differ between the groups. Serum TTR was significantly higher in control than osteoporotic subjects (89.47 and 144.53 µg/ml, respectively, p=0.003, Mann-Whitney U test). TTR was positively correlated with BMD at several sites, such as the total radius bone (r=0.172, p=0.008, Spearman rank test). Osteoporosis risk was analyzed with binary logistic regression. Lean elderly Thais with lower TTR levels had a higher risk of osteoporosis. RBP4 and retinol levels had no relationship with disease status among Thai post-menopausal women. These results suggest calcium, minerals, vitamins and the retinol transport protein, transthyretin may be involved in the pathogenesis of osteoporosis.

Keywords: transthyretin, vitamin A, retinol binding protein 4, post-menopausal Thai women

Correspondence: Dr Rungsunn Tungtrongchitr, Department of Tropical Nutrition and Food Science, Faculty of Tropical Medicine, Mahidol University, 420/6 Ratchawithi Road, Ratchathewi, Bangkok 10400, Thailand.
Tel: 66 (0) 2354 9100 ext 2081; Fax: 66 (0) 2644 7934
E-mail: tmrtg@mahidol.ac.th

INTRODUCTION

The association between vitamin A and osteoporosis is controversial. Some studies have shown high vitamin A intake or high serum retinol may increase the risk for fractures and susceptibility to osteoporosis by reducing bone mineral density

(BMD) (Michaëlsson *et al*, 2003; Maggio *et al*, 2006; Caire-Juvera *et al*, 2009). Some studies have shown no association between vitamin A intake and risk for hip or other fractures in postmenopausal women (Caire-Juvera *et al*, 2009) and among both sexes with age-matched controls (Vestergaard *et al*, 2010).

In the circulation, retinol is transported to target tissues by retinol binding protein 4 (RBP4). This protein is secreted by the liver and adipocytes and has been reported as a new adipokine, playing a role in insulin resistance among obese subjects (Frey *et al*, 2009). A previous study found a negative association between retinol and RBP4 and bone formation markers such as osteocalcin, but not with BMD (Högström *et al*, 2008). RBP 4 levels have been found to be lower in osteoporotic elderly subjects (Rico *et al*, 1995).

To prevent loss of RBP4 and retinol in the kidneys due to its low molecular weight (approximately 21 kDa), it is combined with a more complex 55 kDa protein called transthyretin (TTR), or previously called prealbumin (Zanotti and Berni, 2004). This protein transports thyroxin and is a malnutrition marker (Ingenbleek and Young, 1994). TTR has a significant correlation with osteoporosis in the elderly (Rico *et al*, 1995) and in post-menopausal women subjects (Rico *et al*, 1993). However, the association between retinol, retinol binding protein and transthyretin and osteoporosis is unclear. The aim of the present study was to examine the relationships between retinol, RBP4, TTR, and bone mineral density among Thai post-menopausal women with and without osteoporosis.

MATERIALS AND METHODS

Subjects

Thai post-menopausal women who attended the menopause post-operation follow-up clinic, at the Department of Obstetrics and Gynecology, Ramathibodi Hospital, Bangkok, for a physical examination and bone scan (DEXA) were recruited. Seventy-three osteoporotic and 71 normal women age >50 years were included in the study. They were classified by T-score for BMD in the lumbar spine, hips or radius using dual-energy X-ray absorptiometry (DEXA) (Lunar Prodigy, New York, NY). Women who had a T-score ≤ -2.5 SD below normal were regarded as osteoporotic. A T-score ≥ -1.0 SD were considered normal (WHO, 2003). All subjects gave informed consent prior to participation in the study. Exclusion criteria was the presence of a disease known to affect bone metabolism. A physical examination was conducted by a medical doctor in the study. The study protocol was approved by the Ethics Committee of the Faculty of Tropical Medicine and of Faculty of Medicine at Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.

Biochemical measurements

The serum concentrations of RBP4 and TTR were quantified by non-commercial enzyme-linked immunosorbent assays (ELISA) (Raila *et al*, 2007). Serum vitamin A (retinol) was measured by a reverse-phase high performance liquid chromatography (RP-HPLC) as described previously (Schweigert *et al*, 2003).

Data analysis

Median, range and 95% confidence intervals for the median were calculated. The Mann-Whitney *U* test was used to evaluate differences in proportions between osteoporotic and control subjects. The Spearman rank test was used for correlation analysis. Binary logistic regression was used to evaluate the risk for osteoporosis of the combined factors.

Table 1
Characteristics of controls and osteoporotic subjects.

	Osteoporosis value (95%CI)	Control value (95%CI)
Age (years)	62.00 (60.00-63.00)	53.00 (52.00-54.00)
BMI (kg/m^2)	21.38 (20.83-22.01)	25.01 (23.73-26.20)
Waist hip ratio	0.81 (0.79-0.82)	0.82 (0.81-0.84)
Total body BMD (g/cm^2)	1.011 (0.990-1.022)	1.184 (1.154-1.225)
L1-4 BMD (g/cm^2)	0.896 (0.861-0.926)	1.246 (1.178-1.271)
Total radius BMD (g/cm^2)	0.413 (0.413-0.431)	0.541 (0.533-0.562)
Total hip BMD (g/cm^2)	0.791 (0.772-0.820)	1.050 (0.993-1.081)
Femoral neck (g/cm^2)	0.724 (0.706-0.745)	0.915 (0.872-1.013)

95% CI, 95% confidence interval for the median.

Table 2
Biochemical data in osteoporotic and control subjects.

Biochemical data (mg/dl)	Osteoporotic subjects		Control subjects		*p*-value
	Median	95% CI	Median	95% CI	
Albumin	45.0	44.2 - 45.8	44.8	44.3 - 44.5	NS
Calcium	9.2	9.2 - 9.3	9.4	9.3 - 9.5	0.041
Glucose	92.0	88.0 - 94.0	91.0	90.0 - 96.0	NS
Triglycerides	87.0	81.0 - 97.6	86.5	75.15 - 104.43	NS
Total cholesterol	229.0	209.0 - 234.0	211.5	200.7 - 222.0	NS
HDL-cholesterol	59.0	52.0 - 63.0	54.0	49.6 - 58.4	NS
LDL-cholesterol	140.0	126.4 -155.2	133.0	124.9 - 152.2	NS

95% CI, 95% confidence interval for the median; p <0.05 was considered statistically significant.

A p-value <0.05 was considered statistically significant. All analyses were carried out using the software program SPSS 11.0 for Windows (SPSS, Chicago, IL) and MINITAB.

RESULTS

Anthropometric data for the osteoporosis and control subjects is shown in Table 1. The median serum calcium level was significantly lower among osteoporotic subjects (Table 2). The HDL-cholesterol level was slightly lower among osteoporotic subjects but the difference was not statistically significant. The other biochemical data did not differ between the subject groups.

The median serum TTR level was significantly higher among control subjects (89.47 and 144.53 μg/ml, p = 0.003). The RBP4 and retinol levels were slightly different between the groups, but the difference was not significant (Table 3). Serum TTR were positively correlated with BMD in the total radius (r = 0.172, p = 0.008, Spearman rank test) (Fig 1). The RBP4 and retinol levels had no correlation with the BMD. Osteoporosis risk was analyzed by binary logistic regression (Table 4). Elderly with a lower BMI and

Table 3
Retinol binding protein 4 (RBP4), transthyretin (TTR), and retinol levels in osteoporotic and control subjects.

Biochemical data (μg/dl)	Osteoporotic subjects		Control subjects		*p*-value
	Median	95% CI	Median	95% CI	
RBP4	41.97	38.06 - 44.28	40.12	37.35 - 43.58	NS
TTR	89.47	79.79 - 100.58	144.53	106.21 - 171.87	0.003
Retinol	0.46	0.440 - 0.500	0.47	0.444 - 0.499	NS

$p < 0.05$ was considered statistically significant.

Table 4
Logistic regression analysis of osteoporosis risk when osteoporotic cutoff was used as a dependent variable, and RBP4, TTR, retinol, calcium, BMI, and age were independent variables.

Variables	B	SE	Wald	df	Odds ratio	95%CI of odds ratio	*p* -value
RBP4 (>42 μg/ml)	-0.256	0.743	0.119	1	0.774	0.180 - 3.320	NS
TTR (>140 μg/ml)	-2.128	0.759	7.854	1	0.119	0.027 - 0.527	0.005
Retinol (μg/ml)	0.494	0.676	0.533	1	0.774	0.180 - 3.320	NS
Calcium (mg/dl)	-1.417	0.826	2.941	1	0.243	0.048 - 1.224	NS
Age (years)	-0.256	0.743	0.119	1	1.357	1.182 - 1.559	<0.000
BMI (kg/m^2)	-0.418	0.122	11.759	1	0.658	0.518 - 0.836	0.001

B, regression coefficient for each predictor variable in the model.
SE, standard error of mean.

a TTR level showing mild-malnutrition (<140 μg/ml) had an increased risk of osteoporosis (odds ratio for TTR: 0.119, $p = 0.005$). RBP4 and retinol levels were not correlated with osteoporosis among Thai post-menopausal women.

DISCUSSION

Osteoporosis is a metabolic disease of the bones affecting many millions of people worldwide. It is characterized by low BMD, an imbalance of bone tissue, increased bone fragility and an elavated fracture risk (Prentice, 2004). Osteoporosis can be diagnosed by determining BMD (Lane, 2006). Nutritional deficiencies are important factors in the development of osteoporosis (Rizzoli *et al*, 2001); however, the link between osteoporosis and vitamin A and its transport protein is unclear. Therefore, the present study investigated the association between RBP4 and transthyretin and BMD in osteoporotic Thai post-menopausal women.

TTR is an indirect retinol carrier because it is bound to RBP4 (Ingenbleek and Young, 1994). Alteration in protein and calorie intake can be assessed by measuring TTR in the blood (Spiekerman, 1993). In contrast to serum albumin, the half-life of transthyretin is relatively short, only 2-3

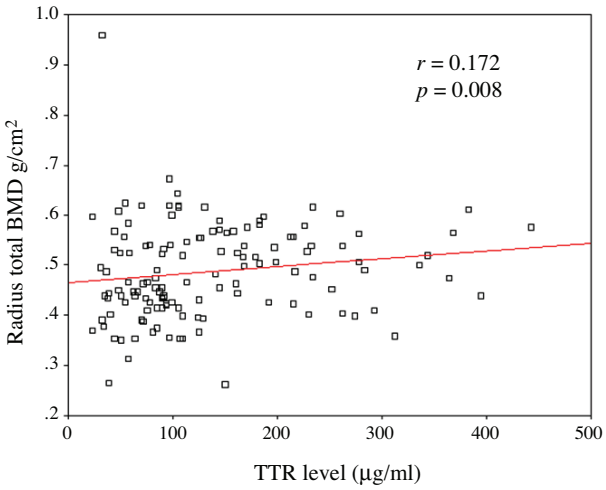

Fig 1–Correlation between TTR level (µg/ml) and radius BMD (g/cm^2).

days. It may be a more sensitive indicator of nutritional status than serum albumin (Mittman *et al*, 2001).

In this study, we found significantly lower TTR levels in osteoporotic women than in control subjects, which corresponds to previous studies (Rico *et al*, 1993, 1995). A positive correlation was seen between TTR levels and radius total BMD. Besides calcium, minerals, and vitamins (Rico *et al*, 1995). TTR protein is a nutritional protein marker and may be associated with osteoporosis. TTR is a marker for protein loss frequently found in renal disease. Retinol concentration was not associated with BMD at any site, similar to a previous study (Högström *et al*, 2008). These results are in contrast with those of Maggio *et al* (2006) who found retinol was lower in osteoporotic subjects than controls. Several studies reported lowers levels of retinol were a risk factor for fracture (Michaëlsson, 1999, 2003).

RBP4 levels were the same in osteoporotic and control subjects and were not correlated with BMD, similar to other studies (Ballew *et al*, 2001; Hogstrom *et al*, 2008). However, RBP4 and TTR levels had a positive association with retinol. Logistic regression analysis showed elderly subjects with a low BMI and low TTR level, were an increased risk for osteoporosis, but retinol and RBP4 were not associated (Ersoy *et al*, 2006; Caire-Juvera *et al*, 2009). Serum calcium levels differed between the subject groups, but this result disappeared when age and BMI were included in the calculation.

Age had a negative correlation with TTR level (data not showed), similar to previous studies (Rico *et al*, 1995, Rambod *et al*, 2008), suggesting aging is associated with increased risk for a poor diet. This is an important factor in the development of osteoporosis. Further studies should include markers for bone metabolism to determine their relationship with TTR, RBP4 and retinol in osteoporosis.

In conclusion, this study showed lower TTR, BMI and serum calcium in osteoporotic patients than controls. Malnutrition is a risk factor for osteoporosis and TTR may be a marker for osteoporosis. RBP4 and retinol had no association with BMD in Thai post-menopausal women.

ACKNOWLEDGEMENTS

The authors wish to thank all of the volunteers; Andrea Hurtienne and Uldine Ulrich for their expert technical assistance; and the staff of the Institute of Nutritional Science, University of Potsdam for their cooperation in this research. This work was supported by a Mahidol University Research Fund, the Royal Golden Jubilee PhD program under the Thailand Research Fund (TRF), the Faculty of Tropical Medicine, and a NRU grant through the Center for Biopharmaceutical Development and Innovative Therapy, Mahidol University.

REFERENCES

Ballew C, Galuska D, Gillespie C. High serum retinyl esters are not associated with reduced bone mineral density in the Third National Health and Nutrition Examination Survey, 1988-1994. *J Bone Miner Res* 2001; 16: 2306-12.

Caire-Juvera G, Ritenbaugh C, Wactawski-Wende J, Snetselaar LG, Chen Z. Vitamin A and retinol intakes and the risk of fractures among participants of the Women's Health Initiative Observational Study. *Am J Clin Nutr* 2009; 89: 323-30.

Ersoy FF, Passadakis SP, Tam P, *et al*. Bone mineral density and its correlation with clinical and laboratory factors in chronic peritoneal dialysis patients. *J Bone Miner Metab* 2006; 24: 79-86.

Frey SK, Spranger J, Henze A, Pfeifferb AFH, Schweigert FJ, Raila J. Factors that influence retinol-binding protein 4-transthyretin interaction are not altered in overweight subjects and overweight subjects with type 2 diabetes mellitus. *Metab Clin Exp* 2009; 58: 1386-92.

Högström M, Nordström A, Nordström P. Retinol, retinol-binding protein 4, abdominal fat mass, peak bone mineral density, and markers of bone metabolism in men: the Northern Osteoporosis and Obesity (NO_2) Study. *Eur J Endocrinol* 2008; 158: 765-70.

Ingenbleek Y, Young V. Transthyretin (prealbumin) in health and disease: nutritional implications. *Annu Rev Nutr* 1994; 14: 495-533.

Lane NE. Epidemiology, etiology, and diagnosis of osteoporosis. *AmJ Obstet Gynecol* 2006; 194: S3-11.

Maggio D, Polidorib C, Barabania M, *et al*. Low levels of carotenoids and retinol in involutional osteoporosis. *Bone* 2006; 38: 244-8.

Michaëlsson K, Holmberg L, Mallmin H, *et al*. Diet and hip fracture risk: a case-control study. *Int J Epidemiol* 1995; 24: 771-82.

Michaëlsson K, Lithell H, Vessby B, Melhus H. Serum retinol levels and the risk of fracture. *N Engl J Med* 2003; 348: 287-94.

Mittman N, Avram MM, Oo KK, Chattopadhyay J. Serum prealbumin predicts survival in hemodialysis and peritoneal dialysis: 10 years of prospective observation. *Am J Kidney Dis* 2001; 38: 1358-64.

Prentice A. Diet, nutrition and the prevention of osteoporosis. *Public Health Nutr* 2004; 7: 227-43.

Raila J, Henze A, Spranger J, Mohlig M, Pfeiffer AFH, Schweigert FJ. Microalbuminuria is a major determinant of elevated plasma retinol-binding protein 4 in type 2 diabetic patients. *Kidney Int* 2007; 72: 505-11.

Rambod M, Kovesdy CP, Bross R, Kopple JD, Kalantar-Zadeh K. Association of serum prealbumin and its changes over time with clinical outcomes and survival in patients receiving hemodialysis. *Am J Clin Nutr* 2008; 88: 1485-94.

Rico H, Relea P, Crespo R, *et al*. Biochmical markers of nutrition in type-I and type-II osteoporosis. *J Bone Joint Surg* 1995; 77: 148-51.

Rico H, Relea M, Hernandez ER, Arribas I, Villa LF. Biochemical markers of nutrition in osteoporosis. *Calcif Tissue Int* 1993; 52: 331-3.

Rizzoli R, Ammann P, Chevalley T, Bonjour JP. Protein intake and bone disorders in the elderly. *Joint Bone Spine* 2001; 68: 383-92.

Schweigert FJ, Steinhagen B, Raila J, Siemann A, Peet D, Buscher U. Concentrations of carotenoids, retinol and alpha-tocopherol in plasma and follicular fluid of women undergoing IVF. *Hum Reprod* 2003; 18: 1259-64.

Spiekerman AM. Proteins used in nutritional assessment. *Clin Lab Med* 1993; 13: 353-69.

Vestergaard P, Rejnmark L, Mosekilde L. High-dose treatment with vitamin A analogues and risk of fractures. *Arch Dermatol* 2010; 146: 478-s82.

World Health Organization (WHO). Prevention and management of osteoporosis. *World Health Organ Tech Rep Ser* 2003; 921: 57-62.

Zanotti G, Berni R. Plasma retinol-binding protein: structure and interactions with retinol, retinoids, and transthyretin. *Vitam Horm* 2004; 69: 271-95.