Serum retinol level and osteoporosis in postmenopausal women with type 2 diabetes mellitus

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SOUHRN

Raška Jr. I., Rašková M., Vojtová L., Zikán V.: **Hladina retinolu v séru a osteoporóza u postmenopauzálních žen s diabetem mellitem 2. typu**

Úvod: Pacienti s diabetem mellitem 2. typu (DM2) mají zvýšené riziko zlomenin ve srovnání s jedinci bez DM2 nezávisle na hustotě kostního minerálu (BMD). Stále chybějí biochemické parametry, odrážející špatnou kvalitu kosti u DM2. Plně pochopena není role vitamínu A v patogenezi křehkosti kostí u populace s diabetem.

Cíl: Cílem naší studie bylo zjistit hladinu retinolu v séru a zkoumat vztah mezi hladinou retinolu v séru a metabolismem kostí nebo glukózy u postmenopauzálních žen s DM2 a kontrolních subjektů.

Design a metody: Provedli jsme průřezovou observační studii u 57 postmenopauzálních žen s DM2 a 73 postmenopauzálních žen bez DM2. Hladiny retinolu v séru byly měřeny pomocí vysokoúčinné kapalinové chromatografie (HPLC). Ke stanovení BMD byl použit dvouenergetický absorpční denzitometr (Discovery A, Hologic Inc, USA, software Apex 3.0).

Výsledky: Nezjistili jsme významné rozdíly v hladině retinolu v séru mezi postmenopauzálními ženami s DM2 (průměrná hodnota 0,43 ± 0,2 mg/l) a kontrolními subjekty (průměrná hodnota 0,36 ± 0,1 mg/l). Ženy s osteoporózou a DM2 měly významně vyšší hladinu retinolu v séru než kontrolní subjekty s osteoporózou ($p < 0,05$). Nebyl zjištěn žádný vztah mezi hladinou retinolu v séru a BMD, výskytem zlomenin, BMI, trváním diabetu, glukózou nalačno nebo glykovaným hemoglobinem u žen s DM2.

Závěr: V naší studii hladina retinolu v séru nesouvisí s výskytem zlomenin, BMD nebo parametry metabolismu glukózy u postmenopauzálních žen s DM2. Avšak vyšší hladina retinolu u postmenopauzálních žen s DM2 připouští možný podíl vitamínu A na patogenezi úbytku kostní hmoty u postmenopauzálních žen s DM2. Potřebné jsou další studie zkoumající roli vitamínu A v metabolismu kostí u DM2.

Klíčová slova: diabetes mellitus 2. typu, osteoporóza, hustota kostního minerálu, retinol

SUMMARY

Raška Jr. I., Rašková M., Vojtová L., Zikán V.: **Serum retinol level and osteoporosis in postmenopausal women with type 2 diabetes mellitus**

Background: Patients with type 2 diabetes mellitus (T2DM) are at higher risk of fracture compared with non-T2DM individuals independent of bone mineral density (BMD). Biochemical parameters reflecting poor bone quality in T2DM are still missing. The role of vitamin A in the pathogenesis of bone fragility in diabetic population is not fully undestood.

Objective: The objectives of our study were to assess the serum retinol level and to investigate the relationship between serum retinol level and bone or glucose metabolism in T2DM postmenopausal women and control subjects.

Design and methods: We conducted a cross-sectional observational study in 57 postmenopausal women with T2DM and 73 postmenopausal women without T2DM. Serum retinol levels were measured using high-performance liquid chromatography (HPLC). BMD was determined using a dual energy X-ray absorptiometry densitometer (Discovery A, Hologic, Inc, MA, USA, Software vision: Apex 3.0).

Results: We have found no significant difference in serum retinol level between T2DM postmenopausal women (the mean 0.43 ± 0.2 mg/l) and control subjects (the mean 0.36 ± 0.1 mg/l). T2DM women with osteoporosis had significantly higher serum retinol level than control subjects with osteoporosis ($p < 0.05$). No association was found between serum retinol level and BMD, fracture prevalence, BMI, duration of diabetes, fasting glucose or glycated hemoglobin in women with T2DM.

Conclusion: In our study serum retinol level is not related to fracture prevalence, BMD or glucose metabolism parameters in T2DM postmenopausal women. However, higher retinol level in T2DM postmenopausal women with osteoporosis admitt a possible contribution of vitamin A to the pathogenesis of bone loss in postmenopausal women with T2DM. Further studies evaluating the role vitamin A in bone metabolism in T2DM are warranted.

Keywords: Type 2 Diabetes Mellitus, Osteoporosis, Bone Mineral Density, Retinol

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Introduction

After discovering that patients with T2DM have an increased risk of fracture independent of BMD substantial research efforts have focused on studying the bone quality in patients with T2DM. The bone quality results from the bone macro- and microarchitecture, bone remodeling changes and properties of bone matrix. Accumulation of advanced glycation endproducts (AGEs) within the bone induces the formation of covalent cross-links with collagen and other bone proteins which might affect the mechanical properties of bone tissue, and disturbs the bone remodeling [1]. Previous studies have reported decreased levels of osteocalcin [2], lack of vitamin D [3,4] or low bone turnover [5] in T2DM patients. Elevated serum sclerostin, as an osteocyte expressed negative regulator of bone formation [6], has been associated with vertebral fracture in T2DM patients [7]. Despite intensive research to assess the bone quality using noninvasive imaging techniques, biochemical parameters that reflect poor bone quality in T2DM are still missing. To our knowledge, there is a lack of data concerning the relation between vitamin A levels and BMD or fracture risk in T2DM population.

Vitamin A and Bone

In humans, serum retinol level level has been positively associated with dietary vitamin A intake [8,9]. Vitamin A is stored in the liver in the form of retinyl ester and mobilized from the liver as retinol, normaly bound to retinol binding protein [10]. Retinol is released in target cells and converted to retinoid acid, which exerts its effect binding to specific nuclear receptors [11]. It is known that excess of vitamin A intake can result in adverse skeletal effects by suppressing osteoblast activity and promoting osteoclast formation thereby stimulating bone resorption and inhibiting bone formation [12]. Vitamin A was found as a negative factor of osteoblast mineralization [13]. In our previous study we have reported that slight excess of all-trans-retinoic acid (ATRA) leads to significant bone loss in both intact and castrated mice [14]. Studies concerning the relationship between vitamin A intake and risk of fracture in non-diabetic population have shown inconsistent results [15–18]. Metaanalysis of prospective studies concerning the impact of vitamin A status on fracture risk have found U-shaped relationship between serum retinol level and hip fracture risk. Both high or low level of serum retinol levels increased the risk of hip fracture [18].

Vitamin A and Type 2 Diabetes Mellitus

Studies have found significantly decreased serum vitamin A level in type 1 diabetes and increased vitamin A level in type 2 diabetes patients compared to non-diabetic controls [19] as well as increased vitamin A level in subjects with impaired glucose tolerance compared to controls [20]. Later, other studies have shown an inverse association between carotenoids and plasma glucose concentrations [21]. More recent study has found, that vitamin A is required for both maintenance of pancreatic beta- and alpha-cell mass and for glucose stimulated insulin secretion in adult mice. Dietary vitamin A deprivation caused greatly decreased pancreatic vitamin A level, hyperglycemia and reduced insulin secretion in adult mice [22].

The objectives of our study were to assess the serum retinol level and to investigate the relationship between serum retinol level and BMD or glucose metabolism indices in T2DM postmenopausal women and control subjects.

Material and Methods

Patients

Postmenopausal women with T2DM on oral anti-diabetic medication or newly detected T2DM attending a preventive bone mineral density (BMD) measurement from October 2012 till October 2014 were considered for the study. Exclusion criteria were abnormal serum calcium level, serum creatinine level > 110 μmol/l, serum CRP elevation, diseases other than osteoporosis and T2DM that would interfere with bone metabolism such as liver disease, malabsorption, diabetic nephropathy, primary hyperparathyroidism; or use of any other medication affecting bone metabolism within the 3 years prior the selection, such as bisphosphonates, raloxifene, strontium ranelate, fluoride, hormone replacement therapy, glucocorticoids, thiazolidinedions, insulin, vitamin A supplements or vitamin D supplements. A total of 57 postmenopausal women with T2DM (mean age 66.5 ± 7 years) were eligible for the analysis. The control group consisted of postmenopausal women without T2DM who attended a preventive bone mineral density (BMD) measurement. Respecting the same exclusion criteria also for the control group, a total of 73 postmenopausal women without T2DM (mean age 65.1 ± 9 years) were eligible for the study. The study was undertaken with the understanding and written consent of each subject, with the approval of the Ethics Committee of the General University Hospital, and within compliance of the National Legislation and the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association (Declaration of Helsinki).

Bone Mineral Density Measurement

BMD was determined using a dual energy X-ray absorptiometry (DXA) densitometer (Discovery A, Hologic, Inc, MA, USA, Software vision: Apex 3.0). BMD was measured

at the lumbar spine (L1–L4), total femur, femoral neck, distal radius and whole body in all participants. Osteoporosis was defined as T-score ≤ –2.5 SD, osteopenia as T-score between –1 and –2.5 SD and normal bone mineral density as T-score ≥ –1 SD.

Laboratory Analysis

Venous blood samples were taken after a 12 hour overnight fast. Routine biochemical analyses were performed with fresh samples; other aliquots were stored at –70 ºC before being analyzed. The serum calcium levels were measured by the standard automated analytical procedure (Modular; Roche Diagnostics, Germany). The serum glycated hemoglobin (HbA1c) concentrations were assessed by high performance liquid chromatography. The serum fasting glucose was measured by enzymatic colorimetric (GOD-PAP) method. The serum retinol concentrations were measured by high-performance liquid chromatography (HPLC, Agilent Technologies 1260) after previous preparation using Retinol A separation kit (ClinRep Complete Kit for Vitamins A and E in Plasma, RECIPE). Normal serum retinol range was 0.3–0.6 mg/l (1.05–2.10 umol/l). Severe vitamin A deficiency was defined as serum retinol level < 0.1 mg/l (0.35 umol/l) and levels > 1.4 mg/l (4.9 umol/l) were considered as toxic [23].

Statistical analysis

Data were expressed by means and standard deviations if not otherwise stated. T test or ANOVA with Tukey test for multiple comparison procedures was used to determine differences between groups. The associations were analyzed using multiple linear regression analysis. The significance was reached with a p value < 0.05. Statistical analyses were made using the SigmaStat statistical software v.3.5 (Jandel, San Rafael, USA).

Results

The demographic data and patients characteristic are stated in *Table 1*. We have found no significant difference in serum retinol level between T2DM postmenopausal women (the mean 0.43 ± 0.2 mg/l) and control subjects (the mean 0.36 ± 0.1 mg/l). A total of 12 T2DM women (21 %) had serum retinol level below normal range (< 0.3 mg/l), 34

Table 1
The demographic data and patients characteristics

	Postmenopausal T2DM Women	Controls	p value
N	57	73	
Age (years)	66.5 ± 7	65.1 ± 9	ns
Years after menopause (years)	17.9 ± 11.4	17.5 ± 12.2	ns
BMI (kg/m^2)	32.1 ± 8.1	26.3 ± 6.6	< 0.001
S-retinol (mg/l)	0.43 ± 0.2	0.36 ± 0.1	ns
S-calcium (mmol/l)	2.3 ± 0.1	2.3 ± 0.1	ns
S-creatinine (µmol/l)	68.6 ± 14	71.2 ± 10	ns
eGFR (ml/s/1,73m^2)	1.20 ± 0.21	1.17 ± 0.16	ns
S-fasting glucose (mmol/l)	6.9 ± 1.5	5.2 ± 0.6	< 0.001
S-HbA_{1c} (mmol/mol)	51.7 ± 14	38.5 ± 4	< 0.001
BMD Lumbar spine (g/cm^2)	0.95 ± 0.15	0.86 ± 0.14	< 0.001
BMD Total femur (g/cm^2)	0.90 ± 0.13	0.82 ± 0.15	< 0.001
BMD Femoral neck (g/cm^2)	0.72 ± 0.11	0.67 ± 0.09	< 0.01
BMD Distal Radius (g/cm^2)	0.61 ± 0.07	0.58 ± 0.08	ns
Duration of T2DM (years)	6.58 ± 6.4	–	
Forearm fracture	9 (16%)	11 (15%)	
Hip fracture	0 (0%)	0 (0%)	
Vertebral fracture	6 (11%)	10 (14%)	

Abbreviations: BMI – Body Mass Index, eGFR – estimated Glomerular Filtration Rate, HbA_{1c} – glycated hemoglobin A1c BMD – Bone Mineral Density, T2DM – Type 2 Diabetes Mellitus

Table 2
Bone mineral density and glucose metabolism parameters in postmenopausal women with type 2 diabetes mellitus divided according to serum retinol level

Serum retinol level	< 0.3 mg/l	0.3–0.6 mg/l	> 0.6 mg/l	p value
N	12	34	11	
Age (years)	65.8 ± 7.4	67.1 ± 7.4	65.6 ± 6.1	ns
BMI (kg/m^2)	32.6 ± 6.9	31.4 ± 9.8	33.5 ± 5	ns
S-creatinine (μmol/l)	69.18 ± 15.1	69.38 ± 15.3	65.9 ± 8.9	ns
eGFR (ml/s/1, 73 m^2)	1.21 ± 0.22	1.36 ± 0.26	1.25 ± 0.13	ns
S-fasting glucose (mmol/l)	7.53 ± 1.5	6.65 ± 1.5	6.8 ± 1.6	ns
Diabetes duration (years)	8.33 ± 7.4	6.82 ± 6.7	3.9 ± 2.0	ns
S-HbA1c (mmol/mol)	51.5 ± 11.6	53.07 ± 17.2	48.1 ± 7.9	ns
BMD Lumbar spine (g/cm^2)	0.91 ± 0.16	0.98 ± 0.15	0.93 ± 0.14	ns
BMD Total Femur (g/cm^2)	0.91 ± 0.12	0.89 ± 0.13	0.92 ± 0.15	ns
BMD femoral Neck (g/cm^2)	0.73 ± 0.11	0.72 ± 0.12	0.72 ± 0.11	ns

Abbreviations: BMI – Body Mass Index, eGFR – estimated Glomerular Filtration Rate, HbA_{1c} – glycated hemoglobin A_{1c} BMD – Bone Mineral Density

T2DM women (60 %) had serum retinol level within normal range (0.3–0.6 ml/l), 11 T2DM women (19 %) had serum retinol levels higher than 0.6. mg/l, the prevalence in the control group was 36 %, 57 %, 7 %, resp. None of T2DM postmenopausal women or control subjects had severe vitamin A deficiency or reached toxic serum retinol levels.

We found no association between serum retinol level and BMI, glycated hemoglobin, fasting glucose or duration of diabetes in T2DM women. Moreover, we have found no significant difference in glucose metabolism indices or BMD in T2DM women with lower or higher serum retinol level in comparison with T2DM women with normal range of serum retinol level (*Table 2*).

In this cross-sectional study, postmenopausal women with T2DM had significantly higher lumbar spine ($p < 0.001$), total femur ($p < 0.001$) as well as femoral neck ($p < 0.01$) BMD than control subjects (*Table 1*). Out of 57 T2DM women 28 % had normal bone mineral density, 47 % osteopenia and 28 % osteoporosis; in a control group 10 %, 37 % and 53 %, resp. However, despite higher BMD we found comparable prevalence of low trauma vertebral and forearm fracture between T2DM patients and control subjects (11 % and 16 % of T2DM women vs. 14 % and 15 % of control subjects, resp.). We found no association between serum retinol level and BMD at any measured region (*Table 1*).

There was also no significant difference in serum retinol level between T2DM women with and without history of prevalent low trauma vertebral or forearm fracture as well as with control group (*Table 3*).

However, T2DM patients with osteoporosis had significantly higher serum retinol level than control subjects with osteoporosis ($p < 0.05$) (*Table 4*).

Discussion

Vitamin A participates in multiple metabolic processes such as genetic expression, cellular differentiation and growth, having an important role in the immune system, fetal development, sight, taste, hearing, appetite and spermatogenesis [24]. Recent studies have shown that vitamin A can also regulate metabolic pathways central to the pathogenesis of T2DM such as pancreatic B-cell function [22,25] or obesity [26]. Retinol binding protein (RBP), a protein that transports retinoids has an important effect on insulin sensitivity, acting as an adipokine [27]. In our study we found no relationship between serum retinol level and BMI, glucose metabolism indices such as glycated hemoglobin, fasting glucose or duration of diabetes in T2DM women. When we analyzed the cohort of T2DM patients according to the serum retinol level we did not find any difference in glucose metabolism parameters as well as in BMI. In agreement with the study of Abahusain [28] we have found also no significant difference in serum retinol level between T2DM patients and control group.

In our study, T2DM women had significantly higher BMI at lumbar spine, total femur as well as femoral neck BMD than control subjects. T2DM is often accompanied with obesity [29] and higher BMD [30]. Several mechanisms explaining this association has been published. Higher skeletal weight-bearing leads to increased BMD through Wnt signaling [31]. Also increased estradiol levels due to increased conversion of androgen precursors to estrogen in the expanded adipose tissue volume may play a role [32].

When focusing on the relationship between serum retinol level and bone metabolism, we did not find any association between serum retinol level and fracture prevalence or BMD

Table 3
Serum retinol level and fracture prevalence in postmenopausal women with and without type 2 diabetes mellitus

	Postmenopausal Women With T2DM			Postmenopausal Women Without T2DM		
	Vfx or Ffx	Without Fx	P value	Vfx or Ffx	Without Fx	P value
N	15	42		19	54	
S-retinol (mg/l)	0.39 ± 0.2	0.44 ±0.2	ns	0.31 ± 0.14	0.38 ± 0.14	ns

Abbreviations: T2DM – Type 2 Diabetes Mellitus, Vfx – Vertebral Fracture, Ffx – Forearm Fracture

Table 4
Serum retinol level and patient characteristics divided according to bone mineral density in postmenopausal women with and without type 2 diabetes mellitus

	Postmenopausal Women With T2DM			Postmenopausal Women Without T2DM		
	Osteoporosis	Osteopenia or Normal DXA	P value	Osteoporosis	Osteopenia or Normal DXA	P value
N	16	41		39	34	
Age (years)	70.6 ± 6	64.9 ± 6	< 0.01	68.4 ± 7	61.6 ± 10	< 0.01
BMI (kg/m^2)	29.4 ± 5	33.11 ± 9	ns	21.7 ± 11	27.0 ± 9	ns
Vertebral Fx	1	5		10	0	
Distal radius Fx	5	4		5	6	
S-Ca (mmol/l)	2.3 ± 0.1	2.3 ± 0.1	ns	2.3 ± 0.1	2.3 ± 0.1	ns
S-creatinine(μmol/l)	69.5 ± 17	68.3 ± 12	ns	71.8 ± 12	68.7 ± 15	ns
S-HbA$_{1c}$ (mmol/mol)	50.7 ± 6	52.1 ±17	ns	39.3 ± 4	37.5 ±4	ns
S-retinol (mg/l)	0.49 ± 0.24 *	0.41 ± 0.19	ns	0.33 ± 0.13	0.4 ± 0.14	ns

Abbreviations: T2DM – Type 2 Diabetes Mellitus, DXA – densitometry, Fx – fracture, BMI – Body Mass Index, eGFR – estimated Glomerular Filtration Rate, HbA$_{1c}$ – glycated hemoglobin A$_{1c}$

*Significant diference in serum retinol level between T2DM women with osteoporosis and postmenopausal women with osteoporosis without T2DM

at any measured region. However, T2DM postmenopausal women with osteoporosis had significantly higher serum retinol level than the control subjects with osteoporosis suggesting a possible role of vitamin A in bone loss in T2DM postmenopausal women. On the other hand, mean values of serum retinol level in both osteoporotic groups with or without T2DM were still within the reference range. Further research is needed to establish the clinical impact of our results. Many questions regarding the role of vitamin A in bone fragility even in non-diabetic population remain unresolved. Previously published studies determining the effect of vitamin A on bone fragility in non-diabetic population brought inconsistent results [15–18]. Moreover, there is no unity in the threshold concentration of serum retinol that increases bone fragility. Based on a study by Michaëlsson, serum retinol level that was higher than 0.86 mg/l (3.0 umol/l) can increase the risk of fracture [10]. In another study, serum retinol level above 0.69 mg/l (2.4 umol/l) was considered to increase fracture risk [33].

Proposed mechanisms showing negative effects of vitamin A on BMD might be in the reduction of bone forming surfaces at the periosteal site as reported in rodents [34] and promoting osteoclast formation thereby stimulating bone resorption and inhibiting bone formation [12]. In our study, to assess bone mineral density we used two-dimensional DXA measurement that does not directly reflect the bone microarchitecture. A novel gray-level texture measurement extracted from DXA images (Trabecular Bone Score) or other advanced imaging techniques such as high resolution peripheral quantitative computed tomography could bring new and better insight into the bone microarchitecture changes linked to vitamin A in humans.

Limitation of the study:

In this cross-sectional study, we did not evaluate the dietary vitamin A intake therefore we could not explore the impact of dietary vitamin A intake on BMD or glucose metabolism parameters. Due to low number of patients in subgroups divided according to their serum retinol level some differences in the investigated parameters could not reach statistical significance. Nevertheless, to our knowledge this is the first study evaluating the relationship between the serum retinol level and bone metabolism parameters in Czech T2DM postmenopausal women.

Conclusion

We found no association between serum retinol levels and glucose metabolism parameters, fracture prevalence or BMD in T2DM postmenopausal women; however, T2DM postmenopausal women with osteoporosis had significantly higher serum retinol level than control subjects with osteoporosis. These findings admitt a possible contribution of vitamin A to the pathogenesis of bone loss in postmenopausal women with T2DM. Further studies evaluating the role of vitamin A in bone metabolism in T2DM are warranted.

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