Journal of Investigative Medicine 56(6):872–877, 2008

ORIGINAL ARTICLE

Renal Injury, Abnormal Vitamin D Metabolism and Bone Homeostasis in Aged Rats With Insulin Resistance or Type 2 Diabetes Mellitus

Huang Chang-Quan, Dong Bi-Rong, Xiao Qian, He Ping, Ding Qun-Fang, Lu Zhen-Chan, and Peng Xiao-Dong

ABSTRACT

Objective: The study aimed to explore the relationship among renal injury, abnormal vitamin D metabolism, and bone homeostasis in insulin resistance (IR) or type 2 diabetes mellitus (T2DM).

Design and Methods: The animal models of IR, T2DM, and T2DM treated with 1-alpha hydroxyvitamin D (1-α(OH)D) were established on 18-month-old male Wistar rats. Glucose infusion rates (GIR) and levels of urinary albumin (UA), serum 25-hydroxyvitamin D (25-(OH)D), serum 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D), and bone mineral density (BMD) in lumbar vertebrae and femoral bone were measured.

Results: Urinary albumin level in the rats with T2DM significantly increased, and there existed a significant and negative correlation between GIR and UA level in the rats with T2DM or IR. The levels of serum 25-(OH)D in all models were similar. The levels of serum 1,25-$(OH)_2$D and BMD in the rats with IR were significantly higher than those in the rats with T2DM and were lower than those in normal control rats. In the aged rats with T2DM, administration of 1-α(OH)D had no effect on serum 25-(OH)D level although significantly increased the levels of serum 1,25-$(OH)_2$D and BMD. There existed a negative correlation between the levels of serum 1,25-$(OH)_2$D and UA in the rats with T2DM or IR.

Conclusions: In IR or T2DM, abnormal vitamin D metabolism is characterized by 1,25-$(OH)_2$D deficiency and is related to renal injury, and there also existed bone loss. In T2DM, both 1,25-$(OH)_2$D deficiency and bone loss can be reversed by 1-α(OH)D.

From the Department of Geriatrics Medicine, West China Hospital, Sichuan University, Chengdu, Sichuan, China.

The authors gratefully acknowledge the financial support of the Medical Science Foundation of Sichuan (070211). We would also like to acknowledge Zhao Ke-Xiang, MD, for assistance in the euglycemic insulin clamp technique; Cheng Gang, MD, for assistance in RIA; and Niu Gui -Fen, MD, for help in dual energy x-ray absorption.

Reprints: Dong Bi-Rong, The Geriatric Department, Westchina Hospital, Sichuan University, Guoxuexiang 37, Chengdu 6100041, China. E-mail: huangshan6662004@yahoo.com.cn.

Key Words: insulin resistance, type 2 diabetes mellitus, vitamin D metabolism, bone mineral density, aged

INTRODUCTION

The course of 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D) production in vivo is mainly regulated by renal 1α-hydroxylase, which is the protein produced in renal tubular epithelial cells.[1–6] 1,25-Dihydroxyvitamin D plays an important role in the regulation of calcium and phosphate metabolism and its deficiency can result in bone loss and osteoporosis.[7] It has been confirmed that in patients with chronic kidney diseases, insufficient renal 1α-hydroxylase is a main factor in accounting for 1,25-$(OH)_2$D deficiency and bone loss.[8,9] Diabetes mellitus and insulin resistance (IR) have been shown to promote the onset and progression of chronic kidney disease and are recognized as the leading cause of end-stage renal disease.[10–15] When considering the relationship between vitamin D and IR or type 2 diabetes mellitus (T2DM), it is generally regarded that abnormal vitamin D metabolism, characterized by 1,25-$(OH)_2$D deficiency, coexists with IR or T2DM and is also recognized as an etiologic factor of IR and T2DM.[16–20] It has been shown that T2DM and IR may increase the risk of bone mineral density (BMD) decrease and fracture.[21–24] Because in the presence of IR or T2DM there exist renal injury, 1,25-$(OH)_2$D deficiency, and disturbance in bone metabolism, it is reasonable to suppose therefore that renal injury with IR or T2DM affects the course of hydroxylation of 25-hydroxyvitamin D (25-(OH)D) in the kidney and leads to 1,25-$(OH)_2$D deficiency, which subsequently result in bone loss. This study intended to investigate the relationship among renal injury, abnormal vitamin D metabolism, and bone homeostasis in the presence of IR or T2DM.

MATERIALS AND METHODS

Preparation of Animal Model

Male Wistar rats (animal-breeding center of Third Military Medical University, Chongqing, China), 18 months of age, each weighing approximately 250 g, were

divided into 4 groups: normal rats (group N; control), IR rats (group I), T2DM rats (group D), and T2DM rats treated with 1α-(OH)D (group T). Each group included 10 rats. Group N was fed with a standard rat diet (gross energy content, 13.35 kJ/g, including 19% fat) for 12 weeks, and in the fourth week, a 3-mL 0.1 mmol/L citrate buffer solution peritoneal injection was administered. Group I received a high-fat diet (gross energy content, 18.71 kJ/g, including 57% fat) for 12 weeks to induce IR and a 3-mL 0.1 mmol/L citrate buffer solution peritoneal injection in the fourth week. Group D received a high-fat diet for 12 weeks and a 30-mg/kg streptozotocin (Sigma Chemical Co, St Louis, MO) peritoneal injection in the fourth week. Group T received the same treatment as group D except that group T received once-daily 0.1 μg/kg 1α-(OH)D (Medicinal Manufacturer of Chongqing, Chongqing, China) via intragastric administration for 4 weeks (from the 8th to 12th week of the experiment). The Institutional Animal Care and Use Committee at Third Military Medical University approved the protocol of this animal experiment.

Body Weight and Fasting Blood Glucose

The rats were weighed at the start and every 4 weeks afterward until the end of the study. Fasting blood glucose (FBG) was determined from tail bleeding at the start and every 4 weeks by the glucose oxidase method[25] with the reagents (Sigma Chemical Co).

IR Detected by Euglycemic Insulin Clamp Technique

At the end of the 12th Week, IR was determined by the use of the euglycemic insulin clamp technique.[26] This method measures the quantity of glucose necessary to compensate for an increased insulin level without causing hypoglycemia. The rats were anesthetized with 3.5% chloral hydrate, using a cannula from the left common carotid and right jugular vein. Insulin (10 $mU \cdot kg^{-1} \cdot min^{-1}$) was infused from the right jugular vein, and arterial blood was collected every 5 minutes from the left common carotid to measure blood sugar levels. When blood sugar levels were 6 ± 0.5 mmol/L, 10% glucose was infused to keep the blood sugar at a level of approximately 6 ± 0.5 mmol/L for 1 hour, and the average glucose infusion rate (GIR) in the hour was calculated. GIR is an index of IR.

UA Detected by Radioimmunoassay

For the measurement of urinary albumin (UA), in the 12th week of the study, the rats were individually housed in metabolic cages and urine was collected over a 24-hour period. The level of UA was determined by an in-house rat albumin radioimmunoassay (RIA)[27] using rabbit antirat albumin antibody RARa/Alb (Amersham Japan, Tokyo, Japan) and globulin-free rat albumin for standard and iodination.

■ 25-(OH)D, 1,25-$(OH)_2$D DETECTED BY RIA

Arterial blood samples were collected for the determination of serum 25-(OH)D and serum 1,25-$(OH)_2$D levels by a RIA kit.

Radioimmunoassay IDS gamma-B25-D-hydroxyvitamin D immunodiagnostic kit from IDS (Amersham Japan) was used for the determination of 25-(OH)D in serum aliquots of 50 μl. In accordance with the information provided by manufacturer, the reference range for such kit is 3 to 83 ng/mL, an analytical sensitivity less than 0.8 ng/mL and a specificity (cross-reactions in %) of sheep polyclonal antibody 100% for 25-(OH)D and less than 0.01% for VD.

1,25-Dihydroxyvitamin D was determined in serum aliquots of 500 μl using the immunoextraction gamma-B1,25-dihydroxyvitamin D RIA kit from the same company. In this case, the immunoextraction is conducted with the aid of monoclonal antibodies immobilized to solid particles. The RIA itself uses a polyclonal sheep antibody whose analytical sensitivity is 8 pg/mL and which shows 100% specificity for 1,25-$(OH)_2$D, less than 0.01% for 24,25-$(OH)_2$D and less than 0.001% for 25-(OH)D. The reference range of 1,25-$(OH)_2$D is 48 to 110 pg/mL.

BMD Detected by Dual Energy X-Ray Absorption

After rats were killed their left femoral bones and lumbar vertebras were collected to detect BMD by using dual energy x-ray absorption (small-animal software high-resolution option, DXA, 4500A; Hologic Inc, Bedford, MA).[28,29]

Statistical Analysis

Data from different groups were combined and reported as the mean ± SD. One-way analysis of variance for multiple comparisons was used to provide a statistical analysis. The relationships among the parameters were analyzed through correlation analysis. All the data were analyzed by the use of the Statistical Package for the Social Sciences (version 12.0; SPSS Inc, Chicago, IL). Values of $P < 0.05$ were considered to be of statistical significance.

■ RESULTS

IR or T2DM in the Aged Rats

At the end of the study, body weight (BW) and increase in BW in group N were lower than those in the other 3 groups (Table 1). At the end of the eighth week, FBG level in each rat in group D and group T was more than 16.7 mmol/L, FBG level in each rat in group I was less than 16.7 mmol/L, but the average FBG level

TABLE 1. FBG, BW, GIR (Mean ± SD, n = 10 per Group) in the 4 Groups: Normal Rats (Group N), Rats With IR (group I), Rats With T2DM (Group D), and T2DM Rats Treated With 1α-(OH)D (Group T)

	Group			
	N	***I***	***D***	***T***
FBG, mmol/L	5.6 ± 2.1*†‡	9.3 ± 3.8†‡§	28.6 ± 9.7¶**	25.3 ± 5.9¶**
BW at start, g	252.6 ± 16.3	248.3 ± 24.6	255.1 ± 17.8	253.9 ± 15.9
BW at end, g	272.4 ± 18.9*††‡‡	302.5 ± 30.7§	297.8 ± 21.4§	293.9 ± 24.5§
Increase in BW, g	19.8 ± 14.2†‡**	54.2 ± 15.7¶	42.7 ± 12.8¶	40.0 ± 16.7¶
GIR, mg·kg^{-1}·min^{-1}	12.3 ± 4.14†‡**	6.8 ± 2.35¶	6.37 ± 1.90¶	8.1 ± 4.54¶

Versus group N, $^{\S}P < 0.05$, $^{\P}P < 0.01$; versus group I, $^{*}P < 0.05$, $^{**}P < 0.01$; versus group D, $^{\dagger\dagger}P < 0.05$, $^{\dagger}P < 0.01$; versus group T, $^{\ddagger\ddagger}P < 0.05$, $^{\ddagger}P < 0.01$.

in group I was higher than that in group N ($P < 0.05$) (Table 1). Group N had a higher GIR than the others ($P < 0.01$), whereas the GIR seen in other groups did not differ significantly (Table 1). These data indicated that there existed IR in groups I, D, and T and T2DM occurred in group D and group T.

UA Level and Its Correlation With GIR in the Aged Rats With IR or T2DM

Groups D and T had higher UA levels than those in group N ($P < 0.01$) and group I ($P < 0.05$); the UA level was higher in group I compared with group N, but the difference was not significant ($P > 0.05$; Table 2). Pearson correlation coefficient showed that UA level negatively and significantly correlated with GIR in groups I, D, and T ($r = -0.8304$, $P < 0.01$; $r = -0.9318$, $P < 0.01$; $r = -0.7743$, $P < 0.05$, respectively) (Fig. 1).

Abnormal Vitamin D Metabolism and Its Correlation With UA Level in the Aged Rats With IR or T2DM

The levels of serum 25-(OH)D in all experimental animals were similar. Serum 1,25-$(OH)_2$D level in the rats with IR was significantly higher than that in the rats with T2DM but significantly lower than that in controls (both $P < 0.05$) (Table 2). This showed that there existed abnormal vitamin D metabolism, characterized by 1,25-$(OH)_2$D deficiency, in the aged rats with T2DM or IR. Pearson correlation coefficient showed that UA level negatively and significantly correlated with level of serum 1,25-$(OH)_2$D for rats across both group I and group D ($r = -0.4946$, $P < 0.05$; $r = -0.7705$, $P < 0.01$, respectively) (Fig. 2).

Bone Loss in the Aged Rats With IR or T2DM

The BMD level in lumbar vertebrae or femoral bone in the rats with IR was lower than that in controls and but higher than that in the rats with T2DM (both $P < 0.05$) (Table 2). This showed that bone loss occurred in the aged rats with IR or T2DM.

The Effect of Administration of 1α-(OH)D on Levels of 1,25-$(OH)_2$D and BMD in the Aged Rats With T2DM

In the rats with T2DM, administration of 1α-(OH)D had no effect on level of serum 25-(OH)D but significantly increased serum 1,25-$(OH)_2$D to a normal level and significantly increased BMD to a level, which was similar to that in the rats with IR (Table 2). In the aged rats with T2DM, reduced levels 1,25-$(OH)_2$D and BMD can be reversed by 1α-(OH)D.

■ DISCUSSION

In the present study in the aged rats with T2DM or IR, there existed abnormal vitamin D metabolism, characterized by 1,25-$(OH)_2$D deficiency and related bone loss. Both 1,25-$(OH)_2$D deficiency and bone loss in the aged rats with T2DM could be reversed by 1-alpha hydroxyvitamin D (1-α(OH)D). For rats with IR or T2DM, UA

TABLE 2. UA, 25-(OH)D, 1,25-$(OH)_2$D, and BMD in Femoral Bone and Lumbar Vertebrae (Mean ± SD, n = 10 per Group) in the 4 Groups: Normal Rats (Group N), Rats With IR (group I), Rats With T2DM (Group D), and T2DM Rats Treated With 1α-(OH)D (Group T)

	Group			
	N	***I***	***D***	***T***
UA, g/L	13.84 ± 4.14*†	18.55 ± 6.52‡§	26.67 ± 9.53¶**	27.0 ± 10.9¶**
25-(OH) D, ng/mL	10.9 ± 4.1	10.7 ± 4.3	10.4 ± 2.9	10.5 ± 2.2
1,25-$(OH)_2$ D, pg/mL	84.38 ± 7.75***	73.1 ± 8.8‡§††	62.4 ± 16.5†¶**	83.2 ± 17.2***
BMD in femoral bone, g/cm^2	0.17 ± 0.04*§**	0.14 ± 0.03‡††	0.12 ± 0.03§¶**	0.15 ± 0.05‡††
BMD in lumbar vertebrae, g/cm^2	0.15 ± 0.05*§**	0.13 ± 0.03‡††	0.10 ± 0.05§¶**	0.13 ± 0.03‡††

Versus group N, $^{\dagger\dagger}P < 0.05$, $^{\P}P < 0.01$; versus group I, $^{**}P < 0.05$, $^{\ddagger\ddagger}P < 0.01$; versus group D, $^{\ddagger}P < 0.05$, $^{*}P < 0.01$; versus group T, $^{\S}P < 0.05$, $^{\dagger}P < 0.01$.

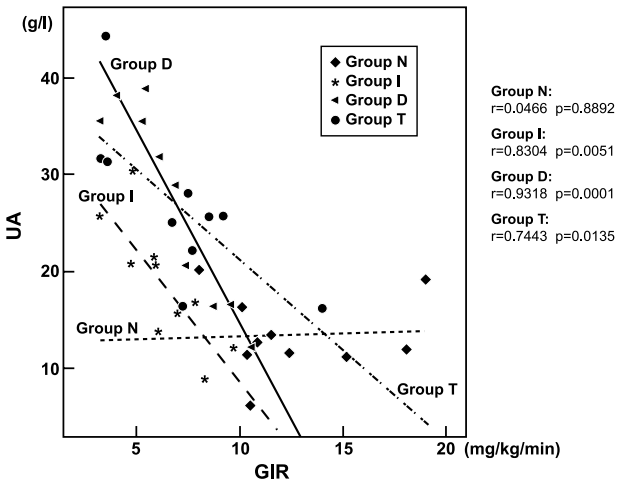

FIGURE 1. Relationship between GIR ($mg \cdot kg^{-1} \cdot min^{-1}$) and UA (g/L) in normal rats (group N), rats with IR (group I), rats with T2DM (group D), and T2DM rats treated with 1α-(OH)D (group T).

level negatively and significantly correlated with GIR and level of serum 1,25-$(OH)_2$D.

25-Hydroxylase converts vitamin D to 25-(OH)D as well as 1-α(OH)D to 1,25-$(OH)_2$D. Renal 1-alpha hydroxylase is necessary in the process of converting vitamin D or 25-(OH)D to 1,25-$(OH)_2$D but is not needed in the process of converting 1α-(OH)D to 1,25-(OH)D.[1–6] In the present study in aged rats with IR or T2DM, there existed normal 25-(OH)D level and reduced 1,25-$(OH)_2$D level. Treating aged rats with T2DM with 1α-(OH)D markedly increased 1,25-$(OH)_2$D to a normal level. Therefore, abnormal vitamin D metabolism, characterized by 1,25-$(OH)_2$D deficiency, occurred in rats with IR or T2DM, and insufficient renal 1α-hydroxylase is the main etiopathogenesis of 1,25-$(OH)_2$D deficiency.[8,9]

The relationship between IR or diabetes and vitamin D has been the subject of research for several decades, and a number of researchers and clinicians believe that abnormal vitamin D metabolism is an etiologic factor for IR and diabetes.[16–20] There are differing viewpoints on whether insufficient 1,25-$(OH)_2$D levels favor IR and diabetes; however, the abnormal vitamin D metabolism characterized by 1,25-$(OH)_2$D deficiency is known to coexist with IR and diabetes.[16–20] There are many reports on insufficient renal 1α-hydroxylase in animal and patients with type 1 diabetes mellitus.[30–32] In this study, we confirmed that in the presence of T2DM or IR, insufficient renal 1α-hydroxylase also occurred and was the main etiopathogenesis of 1,25-$(OH)_2$D deficiency. Thus, we may conclude that diabetes and IR may lead to1,25-$(OH)_2$D deficiency by inducing insufficient renal 1α-hydroxylase.[30–32]

It has been confirmed that renal injury with diabetes and IR is characterized by albuminuria, and the total amount of 24-hour albuminuria can be used as an index of the degree of renal injury with IR or diabetes.[10–15,33–35] In this study, although creatinine and histology change were not measured, we still concluded that renal injury occurred in the aged rats with T2DM from the higher UA level in those rats than that in controls. The aged rats with IR had higher UA level than the controls, but the result was not statistically significant. Considering that UA showed a negative correlation with GIR, this study concluded that the aged rats with IR had renal injury, a hypothesis supported by earlier studies.[34,36] Renal 1α-hydroxylase is a protein that is produced in renal tubular epithelial cells; histological studies on chronic kidney diseases have confirmed that occurrence of microalbuminuria was related to the severe tubular injury.[37–39] In type 1 diabetes mellitus, the effects of insulin deficient and hyperglycemia on kidneys are viewed as mechanism of insufficient renal 1α-hydroxylase.[30–32] Diabetes and IR are the leading cause of chronic kidney disease, and abnormal insulin and hyperglycemia were also viewed as mechanisms of renal injury with IR or diabetes. In this study, in the rats with IR or T2DM, 1,25-$(OH)_2$D deficiency and renal injury coexist, and there were disturbances in glucose metabolism, which had been confirmed as the main reason for both renal injury and insufficient renal 1α-hydroxylase in diabetes.[10–15,30–35] Moreover, there was a correlation between levels of 1,25-$(OH)_2$D and UA in the rats with IR or T2DM. All of these showed that in IR or T2DM, insufficient renal 1α-hydroxylase and 1,25-$(OH)_2$D deficiency were related to renal injury.

In the study, the lower BMD levels showed that there existed bone loss in the aged rats with IR and T2DM. In

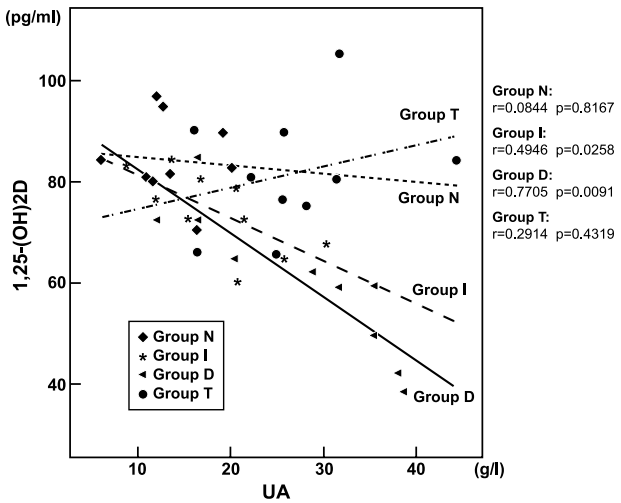

FIGURE 2. Relationship between UA (g/L) and 1,25-$(OH)_2$D (pg/mL) in normal rats (group N), rats with IR (group I), rats with T2DM (group D), and T2DM rats treated with 1α-(OH)D (group T).

the present study, abnormal levels of serum 1,25-$(OH)_2$D, calcium, phosphate, and parathyroid hormone are generally viewed as the reasons for bone loss in diabetes and IR. In the study, we did not measure serum calcium, phosphate, and parathyroid hormone; we are not entitled to make a statement about the effect of serum calcium, phosphate, and parathyroid hormone on bone homeostasis in IR and T2DM. On the other hand, 1,25-$(OH)_2$D deficiency and bone loss were concurrent in the aged rats with T2DM and IR, and both 1,25-$(OH)_2$D deficiency and bone loss were reversed by 1-α(OH)D in the aged rats with T2DM. Moreover, 1,25-$(OH)_2$D deficiency is one of consequent trigger factors of bone loss, which has been demonstrated repeatedly by the clinical and experimental evidences.[8,9,40,41] Thereafter, we can conclude that 1,25-$(OH)_2$D deficiency, which results from insufficient renal 1α-hydroxylase, is a main reason for bone loss in the presence of IR and T2DM.

Based on our results, we concluded here that in senile rats with IR or T2DM, renal injury, abnormal vitamin D metabolism, characterized by 1,25-$(OH)_2$D deficiency, and related bone loss are closely related to one another, and in this case, insufficient renal 1α-hydroxylase may play an important role.

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