Vitamin D homeostasis is compromised due to increased urinary excretion of the 25-hydroxycholecalciferol-vitamin D-binding protein complex in the Zucker diabetic fatty rat

R. L. Anderson,1,2 S. B. Ternes,1 K. A. Strand,1 and M. J. Rowling1,2

1Department of Food Science and Human Nutrition and 2Interdepartmental Graduate Program in Nutritional Sciences, Iowa State University, Ames, Iowa

Submitted 13 April 2010; accepted in final form 22 September 2010

Vitamin D homeostasis is compromised due to increased urinary excretion of the 25-hydroxycholecalciferol-vitamin D-binding protein complex in the Zucker diabetic fatty rat. Am J Physiol Endocrinol Metab 299: E959–E967, 2010. First published September 28, 2010; doi:10.1152/ajpendo.00218.2010.—Altered serum concentrations of the major circulating form of vitamin D [25-hydroxycholecalciferol (25D3)] and its active hormone derivative [1,25-dihydroxycholecalciferol (1,25D3)] have been linked to non-insulin-dependent diabetes mellitus (NIDDM). However, a mechanistic basis for this occurrence has not been fully elucidated. Normally, renal reabsorption of vitamin D-binding protein-bound 25D3 absolutely requires receptor-mediated endocytosis via a receptor complex containing megalin, cubilin, and disabled-2 (Dab2), whereas an absence of megalin or its endocytic partners can lead to a marked urinary loss of 25D and severe vitamin D deficiency. Therefore, we hypothesized that reduced serum vitamin D status in NIDDM may be due to reduced expression of megalin or any of its endocytic partners in NIDDM. This is the first report to our knowledge that associate compromised renal reabsorption of the 25D3-DBP complex with expression of megalin in the kidneys of NIDDM rats. In addition to reduced endocytic partners in NIDDM, which in turn can lead to reduced expression of vitamin D status.

OPTIMAL VITAMIN D STATUS HAS BEEN ASSOCIATED with improved long-term health outcomes in cardiovascular disease and cancer, complications that occur at higher incidences in individuals with non-insulin-dependent diabetes mellitus (NIDDM). Poor renal function, also a consequence of poorly controlled type 2 diabetes, results in hypertension, increased epithelial cell damage, and increased risk for cardiovascular disease (3, 11). In addition to data from case control studies that indicates that maintenance of optimal serum 25-hydroxycholecalciferol (25D3) concentrations (>90 nmol/L) is preventative against many types of cancer, research has suggested that optimal vitamin D status may be protective against hypertension and nephron damage through the suppression of renin production in the kidney (13, 36). Further, more, clinical and epidemiological studies have suggested that type 2 diabetics and individuals with chronic kidney disease are more likely to be in what is considered the suboptimal range (<80 nmol/L) with respect to serum vitamin D status (7, 15), although the mechanistic basis for this occurrence has not been elucidated. Therefore, fully understanding all factors influencing vitamin D homeostasis in this population may reveal opportunities to improve outcomes and comorbidities associated with type 2 diabeties, especially those with renal complications.

Cells of the renal proximal tubule are responsible for reabsorption of the major circulating form of vitamin D (25D3) from the glomerular filtrate and are the primary site of activation of 25D3 to the active hormone 1,25-dihydroxycholecalciferol (1,25D3), which is released into the blood for systemic use. For 25D3 to be reabsorbed and/or activated by CYP27B1 in the kidney, the proximal tubule must internalize the 25D3-vitamin D-binding protein (DBP) complex due to strong binding of DBP to 25D3 in circulation (21, 22). This process is absolutely dependent on the actions of the membrane receptor megalin and its endocytic partners cubilin and disabled-2 (Dab2). In support of this concept, researchers showed that when megalin, cubilin, or Dab2 expression is absent, loss of 25D3-DBP in the urine was dramatically elevated and severe vitamin D deficiency resulted (21, 22). Moreover, renal megalin and cubilin levels were markedly reduced in humans and rats with diabetes (30, 31), which resulted in increased urinary excretion of albumin, another known ligand of megalin.

In the present study, we tested our hypothesis that uncontrolled NIDDM would lead to decreased renal expression of megalin, cubilin, and/or Dab2 in Zucker diabetic fatty (ZDF) rats, a well-established model of NIDDM (34). Furthermore, we hypothesized that reduced expression of megalin or any of its endocytic partners would lead to increased urinary excretion of the 25D3-DBP complex and compromised vitamin D status. Our first objective was to characterize vitamin D homeostasis and expression of megalin, cubilin, and Dab2 in ZDF rats. Second, we determined whether vitamin D deficiency in ZDF rats was due to hormonal changes or reduced endocytosis of the 25D3-DBP complex and whether increased concentrations of cholecalciferol in the diet could influence renal vitamin D reabsorption.

MATERIALS AND METHODS

Animals and Diets

All animal studies were approved by the Institutional Animal Care and Use Committee of Iowa State University and were performed according to Iowa State University Laboratory Animal Resources Guidelines. Male ZDF and lean control rats were obtained from Charles River Laboratories at 6 wk of age and housed...
individually in plastic cages under a 12:12-h light-dark cycle with free access to food and water until 14 wk of age. Study 1: characterization of vitamin D excretion in ZDF rats. ZDF rats (n = 6) and lean rats (n = 6) were fed a commercial, high-energy rodent diet (Purina Formulab Diet 5008) over the course of the study to induce a diabetic state in the ZDF animals and euthanized at 14 wk. Study 2: assessment of vitamin D homeostasis in ZDF rats. Male ZDF (n = 24) and lean (n = 16) rats were fed a high-energy diet (Purina Formulab Diet 5008) until 11 wk, when the ZDF rats were randomly assigned to one of three diets (n = 8), vitamin D deficient (VD; 0 IU cholecalciferol/kg), vitamin D sufficient (VS; 1,000 IU/kg), or vitamin D supplemented (VDS; 10,000 IU/kg), and the lean rats to one of two diet treatments (n = 8), VD or VDS. Diets were formulated on the basis of the AIN-93G purified diet. Animals were euthanized at 14 wk of age.

For both studies, all animals were fasted 24 h prior to euthanization and placed in metabolic cages for urine collection. Animals were anesthetized with an intraperitoneal injection of ketamine-xylazine (90 and 10 mg/kg body wt). Whole kidneys were excised and snap-frozen in liquid nitrogen for subsequent RNA isolation. Whole blood was collected by cardiac puncture, and serum was separated by centrifugation and stored at ~80°C until analysis.

Assessment of Renal Function

Serum and urinary creatinine levels were measured via a commercial kit (QuantChrom Creatinine Assay Kit; Bioassay Systems, Hayward, CA).

Assessment of Blood Glucose and Serum Insulin

To confirm the presence of diabetes in ZDF rats, blood glucose was measured by glucometer (Bayer Healthcare) at the time of euthanization. Serum insulin was analysed using an ELISA specific for rat insulin (Millipore, Billerica, MA).

Assessment of Urinary Albumin and DBP

Urinary albumin and DBP concentrations were measured via commercial ELISA kits (Exocell, Philadelphia, PA, and Life Diagnostics, West Chester, PA, respectively). Sample dilutions used for ELISAs were as directed or empirically determined as appropriate.

Assessment of 25D3 and 1,25D3 Status

Total 25D3 and 1,25D3 were assessed in both serum and urine samples using a commercial enzyme immunoassay kit (Immunodiagnostic Systems, Scottsdale, AZ). Urinary excretion of vitamin D metabolites was assessed relative to urine creatinine.

Real-Time PCR

Total kidney RNA was isolated using SV Total RNA Isolation System (Promega, Madison, WI), quantified by UV detection, and used for first-strand cDNA synthesis (5 μg/50μl reaction) using a High Capacity cDNA synthesis kit with RNase inhibitor (Applied Biosystems, Foster City, CA). Three stocks of cDNA per kidney sample were generated, and each cDNA stock was quantified by UV detection and independently analyzed for megalin, cubilin, Dab2, and CYP27B1 by real-time PCR. Real-time PCR reactions were performed in duplicate using iScript SYBR Green Detection Reagents (Bio-Rad, Hercules, CA), 200 ng cDNA/well, and primer sets specific for rat megalin (forward primer AAGGTCATTGTTTCCGAGCGAA, reverse primer TTGGCAATGCTCATCCTCCACCA), cubilin (forward primer AAGGACACAGGAAACCTTGGCTTA, reverse primer GTCTTTGCTGCATTTGTGGCT), Dab2 (forward primer AGGTGAAAGAAGCCAAACTGCG, reverse primer AGTCTTGCTTTCACCAATCGT, reverse primer CYP27B1, forward primer AGGTGACGTCAGAACATGACC, reverse primer GCTTCGCCCCGGAAGGCXACATCT), and were normalized against 18S mRNA (forward primer CCAGGGCAAGACATTGCGG, reverse primer AATCAAGC-CAAGCTTATGACCCGC). Gene expression was determined as fold induction relative to lean control animals.

Histology and Immunohistochemistry

Formalin-fixed kidneys were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin Y for routine histological assessment. To detect megalin and Dab2, paraffin wax-embedded sections were placed in 1 mol/l urea, microwaved for 10 min, and then cooled for 1 h. Slides were washed three times for 5 min in ddH2O and then washed in phosphate-buffered saline (PBS) for 5 min. Slides were then soaked in 90% methanol containing 30% hydrogen peroxide for 15 min at RT, followed by three washes with PBS (5 min/wash). Following an overnight incubation with blocking buffer (3% bovine serum albumin-0.1% Tween in PBS), slides were incubated overnight with either a 1:50 dilution (in blocking buffer) of a polyclonal antibody directed against megalin (Santa Cruz Biotechnology, Santa Cruz, CA) or a 1:50 dilution (in blocking buffer) of monoclonal antibody directed against Dab2 (BD Pharmingen, San Jose, CA). Slides were then washed three times in PBS and incubated with a 1:500 dilution in blocking buffer of the appropriate biotinylated secondary antibody for 1 h at RT. Following three washes in PBS for 10 min, one drop of ATP-binding cassette (Vector Laboratories, Burlingame, CA) was applied to slides for 30 min at RT. Slides were washed in PBS three times for 5 min, and 3,3-diaminobenzidine tetrahydrochloride (Vector Laboratories) was applied for 5 min followed by a 5-min wash in ddH2O, counterstaining with hematoxylin, and mounting with Permount.

Assessment of Serum Calcium and Parathyroid Hormone

To determine whether vitamin D status was influenced by calcium and parathyroid hormone levels, serum calcium and parathyroid hormone levels were assessed.
Fig. 2. Reduced megalin and disabled-2 (Dab2) expression and renal reabsorption of vitamin D-binding protein (DBP) and albumin in ZDF rats. Renal tissue and urine were collected from the same animals as described in Fig. 1. A and B: megalin and Dab2 mRNA were analyzed as described in MATERIALS AND METHODS. A: megalin mRNA abundance (determined by real-time PCR) in ZDF and lean rats. B: Dab2 mRNA abundance (determined by real-time PCR) in ZDF and lean rats. C and D: urinary DBP and albumin were analyzed as described in MATERIALS AND METHODS. A: urinary DBP concentrations from ZDF and lean rats. B: urinary albumin concentrations from ZDF and lean rats. Data are expressed as means ± SE (n = 6). *P < 0.05; **P < 0.01; ***P < 0.001.

Fig. 3. Renal morphology and immunohistochemical analysis of megalin and Dab2 expression in kidney of lean and ZDF rats. Kidneys were excised from the same animals as described in Fig. 1, processed, and sectioned for staining with hematoxylin and eosin (H & E) for routine histological assessment (top) or subjected to immunohistochemical staining for megalin and Dab2, as described in MATERIALS AND METHODS. Megalin- (middle) and Dab2-positive cells (bottom) appear dark brown against the blue hematoxylin counterstain.
hormone concentrations were measured using commercially available ELISA kits [Bioassay Systems and Immutopics (San Clemente, CA), respectively].

**Statistical Analysis**

Data were analyzed by one-way analysis of variance, unpaired t-test, or Mann-Whitney test using InStat software (version 3.0b for Macintosh; GraphPad Software) or linear regression using Prism software (version 5.0a for Mac OSX; GraphPad Software) as appropriate. Differences between means and linear relationships were considered significant when P values <0.05 were obtained.

**RESULTS**

**Study 1: Characterization of Renal Vitamin D Reabsorption in ZDF Rats**

**Confirmation of NIDDM in ZDF rats.** After 8 wk on the Purina 5008 diet, both fasting blood glucose and serum insulin levels in the ZDF rats were elevated approximately fourfold compared with the nondiabetic control animals (Fig. 1). This confirmed hyperglycemia and hyperinsulinemia in ZDF rats.

**Megalin, cubilin, and Dab2 gene expression.** To determine whether loss of the 25D$_3$-DBP complex in the urine was due to decreased renal absorption, whole kidney lysates were utilized to measure megalin, cubilin, and Dab2 mRNA expression using real-time PCR. We found that both megalin and Dab2 expression was reduced in ZDF animals compared with the lean control animals (~50 and ~80%, respectively; Fig. 2 A and B), whereas we did not detect differences in cubilin mRNA expression (data not shown). Similarly, we found that immunohistochemical staining of tissue sections revealed that megalin and Dab2 protein expression was reduced in the renal proximal tubules in these animals (Fig. 3). Moreover, from our histological observations of kidney tissue from ZDF rats, necrosis appeared to be present in the renal proximal tubules, which may explain, at least in part, the reduced staining of megalin and Dab2 in kidney sections.

**Serum creatinine.** Renal function was assessed by measuring serum creatinine. Serum creatinine levels were elevated ~80% in ZDF animals compared with lean controls, indicating that renal function was compromised in ZDF animals (Fig. 4).

**Albuminuria and increased DBP excretion in ZDF rats.** We measured urinary albumin for two purposes. 1) albumin, like DBP, is a known ligand of megalin (30), and 2) severe albuminuria is a biological marker of nephropathy (8, 30, 31). We found that marked albuminuria was present in the ZDF animals, which excreted ~20-fold greater amounts of albumin compared with lean control animals. Similarly, ZDF rats excreted large amounts of DBP in urine (8.9 µg/ml; Fig. 2, C and D). In contrast, DBP was virtually undetectable in the urine of lean control animals, indicating that the ability of ZDF animals to reabsorb the 25D$_3$-DBP complex was markedly compromised.

**Urinary and serum 25D$_3$ and 1,25D$_3$ concentrations.** Urine was examined for the presence of the 25D$_3$ and 1,25D$_3$ to determine whether loss of DBP in the urine was associated with increased urinary loss of vitamin D. Urine from ZDF rats
animals contained elevated concentrations of total 25D₃ (which is dependent on DBP for transport to the kidney), the major circulating metabolite of vitamin D, compared with lean control animals (31% higher in ZDF animals when normalized to urinary creatinine levels; Fig. 5, B and D). Likewise, urinary concentrations of 1,25D₃ (normalized to urinary creatinine concentrations) were greater in ZDF animals compared with control animals (Fig. 4D). Additionally, urinary DBP concentration strongly correlated with the urinary concentration of 25D₃ (Fig. 6) in the ZDF animals ($r^2 = 0.85, P < 0.05$). Serum 25D₃ and 1,25D₃ levels did not differ between groups (Fig. 5, A and C); however, we determined that consumption of vitamin D per kilogram body weight was significantly higher in ZDF animals (Fig. 7), an indication that loss of urinary vitamin D metabolites was compensated for through the diet, a question that we addressed below in the second set of our experiments.

Study 2: Assessment of Vitamin D Homeostasis in ZDF Rats

Assessment of 25D₃ and 1,25D₃ Status. As in our first set of experiments, NIDDM was confirmed in all ZDF rats. Glucose and insulin levels were 200–400% greater in ZDF rats compared with lean animals (data not shown). After 3 wk of being fed the experimental diet, both 14-wk-old ZDF rats fed a VD (0 IU cholecalciferol/kg) or a VS (1,000 IU cholecalciferol/kg) diet exhibited reduced serum 25D₃ levels compared with their lean counterparts (43 and 22% reduction, respectively) fed the same diets (Fig. 8A). Moreover, urinary excretion of 25D₃ in ZDF animals fed the VD diet was elevated compared with their lean counterparts by 30% after normalization to urinary creatinine (Fig. 8B), indicating that vitamin D status was compromised to a greater extent in the ZDF animals than in lean animals after 3 wk on the VD diet. In support of this concept, urinary excretion of 25D₃ by ZDF rats fed the VS diet was elevated 24% compared with lean rats, and in contrast to study 1, we did not detect differences in cholecalciferol intake per kilogram body weight between the ZDF and lean control animals fed the VS diet (data not shown). Serum 1,25D₃ levels were reduced 70%, whereas urinary 1,25D₃ levels were ~60-fold greater when normalized to urinary creatinine from ZDF animals fed the VD diet compared with lean controls (Fig. 8C), and ZDF animals fed the VS diet exhibited 64% lower serum 1,25D₃ concentrations and excreted approximately ninefold higher concentrations of 1,25D₃ in urine (Fig. 8D). When additional vitamin D (10,000 IU/kg) was added to the diets of the ZDF rats (VDS), large increases were seen in both serum and urinary 25D₃ concentrations (70% and 12-fold, respectively). However, serum concentrations of 1,25D₃ remained markedly lower in ZDF animals fed the VDS diet, indicating that high-dose cholecalciferol supplementation of ZDF rats effectively elevated serum 25D₃ concentrations but had no impact in improving serum 1,25D₃ levels. The latter may be due to the fact that 25D₃ acts as an allosteric inhibitor of CYP27B1 (28), and high serum concentrations of 25D₃ in VDS animals is likely the culprit of low serum 1,25D₃ concentrations in these animals.

Serum Creatinine. As in study 1, renal function was assessed by measuring serum creatinine. Serum creatinine levels were elevated ~76% in ZDF animals compared with lean controls (Fig. 8D), indicating that renal function was compromised in ZDF animals.

Urinary Albumin, DBP, and Renal Megalin Expression in ZDF Rats. Similar to what we observed in our first set of experiments, we detected severe albuminuria and urinary loss of DBP in all ZDF animals compared with their lean controls (Fig. 9, A and B). Urinary albumin concentrations ranged from 470 to 1,250 mg/dl, and urinary DBP concentrations ranged from 2,200 to 3,500 among all ZDF rats, whereas urinary
Fig. 8. Increased urinary output and compromised serum concentrations of 25D₃ and 1,25D₃ in ZDF rats. Eleven-week-old ZDF and lean control rats were fed either 0, 1,000, or 10,000 IU cholecalciferol/kg diet for 3 wk. 25D₃ and 1,25D₃ concentrations were measured in serum and urine collected from ZDF and lean control rats by ELISA, as described in MATERIALS AND METHODS. A: serum 25D₃ concentrations. B: urinary 25D₃ concentrations. C: serum 1,25D₃ concentrations. D: urinary 1,25D₃ concentrations. Data are expressed as means (n = 8) ± SE; bars with different letters are significantly different (P < 0.05). *Statistically significant, ZDF vs. respective lean control (P < 0.05).

Fig. 9. Assessment of megalin expression, renal reabsorption of DBP and albumin, and kidney function in ZDF rats. Renal tissue and urine was collected from the same animals as described in Fig. 7. Renal megalin mRNA expression and urinary DBP and albumin concentrations were determined as described in MATERIALS AND METHODS. A: megalin mRNA abundance in kidney. B: urinary DBP concentrations. C: urinary albumin concentrations. Data are expressed as means ± SE (n = 6); bars with different letters are significantly different (P < 0.05). D: serum creatinine levels of all lean and ZDF animals. Data are expressed as means ± SE (n = 12–18). ***Statistically significant (P < 0.05).
albumin and DBP from lean animals were virtually undetectable. Moreover, as reported above, a reduction in renal mRNA megalin expression was observed in all ZDF groups compared with their lean counterparts (Fig. 9C).

CYP27B1, serum calcium, and parathyroid hormone. Although the diabetic rats all exhibited decreased levels of serum 1,25D₃, serum calcium and parathyroid hormone (PTH) concentrations did not differ between ZDF and lean animals in any of the treatment groups (Fig. 10, A and B). Renal CYP27B1 mRNA expression was elevated only in ZDF animals fed the VD diet, a likely result of reduced serum 1,25D₃ concentrations and/or a lack of dietary cholecalciferol, as we have reported previously (28). Similar to our previously published work (28), a reduction in renal CYP27B1 mRNA expression was observed when ZDF animals were fed the VDS diet.

DISCUSSION

In the present study, we found that NIDDM was associated with increased urinary excretion of 1,25D₃, 25D₃, and DBP, which binds with high affinity >99% of circulating 25D₃ and with low affinity to 1,25D₃ for delivery to the renal proximal tubule for reabsorption (2, 14, 23, 29, 33, 35). Moreover, we found that serum levels of 25D₃ and 1,25D₃ were reduced in animals that not only excreted disproportionately larger amounts of 25D₃, 1,25D₃, and DBP in urine but also exhibited reduced renal expression of megalin and Dab2. Furthermore, we found that compromised vitamin D status was independent of both renal CYP27B1 expression and calcium homeostasis in diabetic animals. Although surprising, our finding that a reduction of serum vitamin D metabolites did not alter calcium homeostasis in ZDF rats may be due to the fact that serum 1,25D₃ levels were not low enough to have an impact on PTH secretion and calcium absorption and homeostasis. Moreover, the concentration of calcium or factors that increase calcium absorption in the purified diet may have contributed to maintaining normal calcium and PTH levels. Regardless, the lack of changes in calcium metabolism in our animal model is independent of our observations that serum 25D concentrations in diabetic animals were reduced, which has important implications with respect to risk of developing secondary chronic diseases in diabetics, such as cancer and autoimmune disorders.

Taken together, our data strongly suggest that the major contributing mechanism behind compromised vitamin D status in our NIDDM animal model was inadequate megalin- and/or Dab2-mediated renal reabsorption of 25D₃ and 1,25D₃. These findings are consistent with a recent clinical study where researchers found that urinary levels of megalin and cubilin were markedly elevated in albuminuric patients with type 1 diabetes (30), further indicating that as diabetes progresses the ability to maintain normal vitamin D homeostasis via megalin-mediated mechanisms is compromised.

A growing number of reports have convincingly linked low vitamin D status to the incidence of a number of chronic diseases, including diabetes, cancers of the colon, breast, and prostate, autoimmune disease, and osteoporosis (6, 19, 32). Additionally, researchers have estimated that the majority of the US population exhibits serum vitamin D levels that are substantially lower than those required for reducing chronic disease risk (6). Hence, interest in utilizing vitamin D in dietary intervention strategies has markedly increased over the last decade. Moreover, the theory that low vitamin D status is a contributing factor to the incidence of chronic disease is at the heart of an ongoing debate about dietary recommendations for vitamin D. On the basis of data from observational studies, Garland et al. (6) recently reported that to provide a 50% reduction in the risk of cancers of the colon and breast, serum levels of 25D₃ need to be ~80 and 110 nM, respectively. It is estimated that, to reach these serum 25D₃ levels, the average individual must consume between 2,000 and 3,500 IU vitamin
D₃/day or 5–7 times the adequate intake defined in the Dietary Reference Intakes for vitamin D₃ (6). In diabetes, the vitamin D requirement for protection against secondary chronic diseases appears to be even greater. This idea is supported by research that demonstrated that type 1 and type 2 diabetics exhibited reduced concentrations of the major circulating form of vitamin D (25D₃) and/or its active derivative 1,25-dihydroxyvitamin D₃ (1,25D₃) (23, 29, 33). There is little known about why vitamin D status is suboptimal in these individuals, but our data suggest that it may be due to compromised kidney function. In support of this concept, we found that rats with NIDDM exhibited a drastic reduction in the renal expression of the endocytic membrane proteins megalin and Dab2, which are essential for reabsorption of protein-bound 25D₃ (30), and increased urinary loss of albumin and DBP, which are also absolutely dependent on megalin for reabsorption. In support of this concept, knockout mouse studies revealed that animals lacking megalin or either of its endocytic partners, cubulin or Dab2, exhibited a marked urinary loss of 25D₃ and severe vitamin D deficiency (17, 18, 21, 22).

Although we and others have found that vitamin D metabolism can be dramatically altered under diabetic conditions (1, 27, 29), exploration of the role of megalin and its endocytic partners in maintaining optimal vitamin status is a new concept. Hence, these studies may offer new insight into whether vitamin D supplementation and monitoring urinary and serum vitamin D levels can help prevent or alleviate secondary complications stemming from compromised kidney function in diabetes. Our findings indicate that reabsorption of vitamin D by the kidney is a major contributing factor to suboptimal vitamin D status in diabetes, which has clear implications with respect to the development of secondary chronic diseases such as cardiovascular disease and many types of cancer. It is well documented that non-insulin-dependent diabetics are at a disproportionately high risk for the development of breast, prostate, and colorectal cancer, cancers that are arguably the most sensitive to the actions of vitamin D. The naturally occurring active form of vitamin D (1,25D₃) has well-documented anti-proliferative actions, including cell cycle arrest, differentiation, and induction of apoptosis (4, 5, 12, 24, 37). Because low serum levels of 1,25D₃ and its precursors are often present in diabetes (1, 14, 27, 29), there may be a substantially larger dietary vitamin D requirement for these individuals. Numerous animal studies have outlined the potential role of increased vitamin D status with respect to the inhibition of tumor formation and promotion. Vitamin D supplementation prior to treatment with chemical carcinogens inhibited fat-induced colorectal tumor promotion in rats fed a high-fat diet (16, 25). Injection of 1,25D₃ or its analogs potently decreased the appearance of aberrant crypt foci and tumors as well as the proliferation and metastasis of established tumors (9). Consistent with this concept, high tumor and polyp frequency as well as increased tumor proliferation have been reported in studies where rodents were fed low-vitamin D diets (10, 20, 26). Furthermore, diabetes has been linked to an increased risk of developing cancer in countless human and animal studies, although the mechanism behind this phenomenon remains unclear. Interestingly, obesity and consumption of a high-fat diet, known risk factors for development of heart disease, cancer, and type 2 diabetes, also appear to strongly affect an individual’s vitamin D requirement (1, 14, 27, 29, 33).

In summary, these studies have provided evidence that megalin-mediated endocytosis plays a critical role in vitamin D homeostasis in NIDDM. Taken together, our data provide the first evidence, at least to our knowledge, that reduced renal reabsorption of circulating vitamin D via compromised receptor-mediated endocytosis is a key contributor to suboptimal vitamin D status in NIDDM.

GRANTS

These studies were made possible by funding granted to M. J. Rowling by the Iowa State University Nutrition and Wellness Research Center/US Department of Agriculture Special Research Grant.

DISCLOSURES

The authors disclose that there is no duality of interest associated with this article.

REFERENCES


