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Basic nutritional investigation

Vitamin K_2 alleviates type 2 diabetes in rats by induction of osteocalcin gene expression

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ABSTRACT

Objectives: The biological mechanisms behind the association between vitamin K (Vit K) and glucose metabolism are uncertain. We aimed to analyze the expression of insulin 1 (*Ins 1*), insulin 2 (*Ins 2*) and *cyclin D2*, the expression of *adiponectin* and UCP-1 . In addition, we aimed to estimate the doses of Vit K2 able to affect various aspects of glucose and energy metabolism in type 2 diabetes.
Methods: Thirty adult male rats were allocated equally into five groups: control group, diabetes mellitus group, and groups 3, 4, and 5, which received Vit K_2 at three daily dose levels (10, 15, and 30 mg/kg, respectively) for 8 wk. At the end of the study, blood samples were collected to quantify total osteocalcin, fasting plasma glucose, fasting insulin, and relevant variables. The expression of *OC*, *Ins 1*, *Ins 2*, *cyclin D2*, *adiponectin*, *UCP-1* genes was analyzed by real-time polymerase chain reaction.
Results: After administration of Vit K_2, a dose-dependent decrease in fasting plasma glucose, hemoglobin A1c and homeostatic model assessment method insulin resistance, and a dose-dependent increase in fasting insulin and homeostatic model assessment method β cell function levels, when compared with diabetes mellitus rats, were detected. There was significant upregulation of *OC*, *Ins 1*, *Ins 2*, or *cyclin D2* gene expression in the three treated groups in a dose-dependent manner when compared with the diabetic rats. However, expression of *adiponectin* and *UCP-1* were significantly increased at the highest dose (30 mg/kg daily) only.
Conclusions: Vit K_2 administration could improve glycemic status in type 2 diabetic rats by induction of *OC* gene expression. Osteocalcin could increase β-cell proliferation, energy expenditure, and *adiponectin* expression. Different concentrations of Vit K_2 were required to affect glucose metabolism and insulin sensitivity.

Introduction

Vitamin K (Vit K) is a fat-soluble vitamin available in two biologically active forms; phylloquinone (Vit K_1) and menaquinones (Vit K_2). Vitamin K_1, the most commonly consumed form, is abundant in green leafy vegetables and certain vegetable oils [1]; whereas Vit K_2 is found in some animal foods and in products derived from bacterial fermentation [1]. Vit K is a multifunctional nutrient and various tissues modulate their function via posttranslational modification of vitamin K-dependent (VKD) proteins according to Vit K bioavailability [2].

One of the typical VKD proteins, osteocalcin (OC), is considered to be the most abundant noncollagenous protein in bone and its concentration in blood is positively correlated with osteoblast function, differentiation, and Vit K availability [3]. Osteocalcin needs VKD posttranslational γ-carboxylation to convert its three Glu residues to Gla residues [3]. During the carboxylation process, a small fraction of OC is uncarboxylated and released into circulation, and high Vit K intakes in a free-living population are associated with a low percentage of undercarboxylated osteocalcin (ucOC) [3]. Earlier studies have indicated the role of OC in the stabilization of bone hydroxyapatite. Recent studies have pointed to the importance of OC as a bone-derived hormone in the regulation of energy expenditure and glucose homeostasis [4]. Osteocalcin increases β-cell proliferation; stimulates insulin expression and secretion; and increases

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expression of adiponectin, an insulin-sensitizing hormone produced by adipocytes [4].

Almost all of the investigations support the hypothesis in rodent models that ucOC secretion from bone restores glucose tolerance through enhanced insulin secretion and sensitivity, or fat metabolism [5–7]. However, in contrast to the rodent models, carboxylated OC (cOC) or total OC (tOC) also may have a close relationship with fat or glucose metabolism in humans [8–10]. Surprisingly, several epidemiologic studies have denoted that higher intake of Vit K as Vit K supplementation has been associated with reduction in insulin resistance and improved glycemic status [11,12]. These findings interfere with the studies postulate that the effects of ucOC improve the glycemic status [13–16]. Therefore, it is likely that Vit K may exert its influence on glycemic status through OC by another mechanism rather than its carboxylation.

It has been reported that Vit K binds to the xenobiotic nuclear receptor (SXR/PXR) leading to enhanced expression of several components of the bone matrix [17,18]. These reports indicated that Vit K is a transcriptional regulator of extracellular matrix–related genes, some of which are involved in collagen assembly [18]. Increased OC levels after Vit K_2 administration might explain the effects of Vit K on osteoblastogenesis [13]. To our knowledge, the effect of Vit K intake on the *OC* expression in patients with diabetes has not yet been studied. In the present study, we hypothesized that Vit K_2 supplementation may ameliorate the glycemic status in type 2 diabetic rats, and if so the mechanism may be by upregulating the expression of *OC*. Also, we embarked on OC signaling pathways of regulation glucose metabolism in a type 2 diabetic rat model. We aimed to analyze the expression of insulin 1 (*Ins 1*), insulin 2 (*Ins 2*), and *cyclin D2* (indicators of β-cell proliferation), the expression of *adiponectin* (indicator of insulin sensitivity) and *UCP-1* (indicator of energy expenditure). In addition we aimed to estimate the doses of Vit K_2 able to affect various aspects of glucose and energy metabolism in type 2 diabetes.

Materials and methods

Drugs and chemicals

Streptozotocin (STZ) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Vitamin K_2 (menatetrenone) was purchased from (Eisai Co., Ltd., Tokyo). All other chemicals used in this study were of analytical grade obtained from Sigma/Aldrich, unless otherwise noted.

Animals

Thirty adult male Wistar rats weighing (200 ± 20 g, 8–10 wk old) obtained from Faculty of Veterinary Medicine, Zagazig University (Egypt) were used. Animals were allowed an acclimatization period for 1 wk at the animal facility of the Faculty of Medicine, Zagazig University. Rats were housed at constant temperature (23 ± 2°C) and a light/dark (12/12 h) cycle. Access to food and water throughout the experimental period was allowed ad libitum. Experimental design and animal handling were in accordance with protocols approved by the institutional research board guidelines of the faculty of Medicine, Zagazig University, which follow the guidelines of the US National Institutes of Health on animal care.

Experimental design

Animals were allocated equally into five groups as follow: Control rats were treated with normal pellet diet for 4 wk then injected with single dose of citrate buffer (pH 4.4, 0.1 M). Diabetic rats were fed a high-fat diet (HFD) consisting 22% fat, 48% carbohydrates, and 20% protein [19]. Fat content was based on olive oil and contained mainly monounsaturated fatty acids, as they have no effects on Vit K_2-dependent processes. After 4 wk on the HFD, the rats were injected intraperitoneally with a single dose of STZ (35 mg/kg) prepared in citrate buffer (pH 4.4, 0.1 M). After 7 d of injection, animals with plasma glucose of ≥300 mg/dL were considered diabetic and included in the study [20]. These animals were further divided into four groups. A diabetic untreated group (DM) was left without any treatment for 8 wk, whereas the remaining three groups received daily Vit K_2 via gavage as follows: 10, 15, and 30 mg/kg for 8 wk [21–23] and were named DM + K10, DM + K15, DM + K30, respectively. Because Vit K_2 is dissolved in corn oil, we added two separate groups: corn oil with citrate buffer and corn oil with STZ. These groups showed an insignificant difference in all the tested parameters from the control and DM groups, respectively, thus we excluded their results. Body weight of all animals was estimated at the end of the study.

Specimen collection

Blood sampling

At the end of the study, animals were anesthetized with urethane (1.3 g/kg) and blood samples were collected from the orbital sinus of rats according to the method of Sorg and Buckner [24]. Animals fasted for a period of 12 h before blood sample collection. Fasting plasma glucose (FPG) levels were measured using glucose oxidase method (Spinreact, Girona, Spain). Hemoglobin A1c was measured by the colorimetric method using a kit obtained from Stanbio Laboratories kit (Boerne, TX, USA). Total cholesterol (TC) and triacylglycerols (TGs) were measured by classic enzymatic methods (spinreact, Girona, Spain). Fasting serum insulin (FSI) and tOC levels were measured using high-sensitivity enzyme-linked immunosorbent assay (ELISA) kit provided by (Biosource Europe S.A., Nivelles, Belgium). Insulin resistance estimation was carried out using homeostatic model assessment method (HOMA-IR) and was calculated using the following formula:

$$\text{FSI } (\mu\text{IU/mL}) \times \text{FPG } (\text{mg/dL})/405.$$

HOMA-B representing β-cell function was calculated using the following formula:

$$[360 \times \text{FSI } (\mu\text{IU/mL})]/[\text{FPG } (\text{mg/dL})–63].$$

Tissue sampling

Rats were sacrificed by decapitation. Pancreas, tibia and femur, white adipocytes (isolated from gonadal fat pads), and brown adipocytes (intrascapular brown fat) were removed and kept at –80°C until RNA extraction.

Tissue homogenization

Tissue samples were homogenized in about 1 mL of TRIzol reagent per 50 to 100 mg of tissue using mortar and pestle with liquid nitrogen. Sample volume did not exceed 10% of the TRIzol Reagent volume was used for homogenization. After homogenization, insoluble material from the homogenate was removed by centrifuging at 10 000 g for 10 min at 4°C. The top layer solution was transferred into a new tube and the pellet was discarded.

RNA extraction, reverse-transcription, and quantification of gene expression

To quantify genes expression, first RNA was isolated from pancreatic, bone, white and brown adipocytes homogenate using the RNeasy Protect Mini Kit (Qiagen, Valencia, CA, USA). RNA (2 μg) was converted into cDNA using the transcriptor first-strand cDNA synthesis kit (Roche, Germany) according to the manufacturer's instructions. Gene expressions were determined by real-time polymerase chain reaction (PCR). Real-time PCR reaction was performed in a total volume of 20 μL containing 5 μL of the cDNA, 100 pmol/μL of each primer (0.5 μL each; Biolegio, Gelderland, The Netherlands), 10 μL of EvaGreen PCR Master mix (Jena Bioscience, Jena, Germany), and 4 μL PCR-grade water. Real-time PCR was performed using Mx3005 P (Stratagene, La Jolla, CA, USA). Amplification reactions were performed using the following cycling conditions and primers that were used for amplification of rat *OC, Ins 1, Ins 2, cyclin D2, adiponectin, UCP-1,* and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) as calibrators are detailed in Table 1. cDNA was replaced by sterile water in the negative controls.

Table 1
Primers sequences for genes and cycling conditions

Gene	Primer sequence	PCR condition	
OC	5′-AAGCCTTCATGTCCAAGCAG-3′	95°C for 10 min	
	5′-TCGTCACATTGGGGTTGAG-3′	95°C for 10 s	40 cycles
		62°C for 10 s	
		72°C for 6 s	
Ins 1	5′GTCCTCTGGGAGCCCAAG 3′	95°C for 10 min	
	5′ACAGAGCCTCCACCAGG 3′	95°C for 10 s	40 cycles
		55°C for 30 s	
		72°C for 8 s	
Ins 2	5′ATCCTCTGGGAGCCCCGC 3′	95°C for 10 min	
	5′AGAGAGCTTCCACCAAG 3′	95°C for 10 s	40 cycles
		55°C for 30 s	
		72°C for 10 s	
Cyclin D2	5′CTGACTGCCGAAAAGCTGTG 3′	95°C for 5 min	
	5′GGGTTACTGCAGCCAGGTTC 3′	95°C for 15 s	40 cycles
		60°C for 30 s	
		72°C for 30 s	
Adiponectin	5′AATCCTGCCCAGTCATGAAG 3′	94°C for 5 min	
	5′CATCTCCTGGGTCACCCTTA 3′	94°C for 15 s	40 cycles
		61.5°C for 15 s	
		72°C for 15 s	
UCP-1	5′-GTGAAGGTCAGAATGCAAGC-3′	94°C for 5 min	
	5′-AGGGCCCCCTTCATGAGGTC-3′	94°C for 45 s	18 cycles
		60°C for 60 s	
		72°C for 60 s	
GAPDH	5′AGTTCAACGGCACAGTCAAG 3′	94°C for 5 min	
	5′TACTCAGCACCAGCATCACC 3′	94°C for 15 s	40 cycles
		60°C for 15 s	
		72°C for 15 s	

Relative quantification of PCR products was determined using the method of $2^{-\Delta\Delta Ct}$. Melting curve analysis was performed to confirm the amplification of a single amplicon for each gene analyzed [25].

Results

Effects of Vit K_2 administration on body weight and biochemical parameters

In the diabetic group, body weight, FPG, HbA1c, HOMA-IR, TC, and TGs increased, whereas fasting insulin and HOMA-B levels decreased compared with the control group. Total OC levels decreased compared with the control group ($P = 0.002$).

A dose-dependent decrease was noticed after daily administration of Vit K_2 at 10, 15, and 30 mg/kg in body weight, FPG, HbA1c, and HOMA-IR compared with the DM group. No significant difference was observed between HOMA-IR scores of any of the three treated groups compared with control ($P > 0.05$). Moreover, a dose-dependent increase in fasting insulin and HOMA-B levels in the DM + K10, DM + K15, and DM + K30 groups was detected when compared with DM rats.

There were no significant changes in TC and TG levels between DM rats and DM rats treated with Vit K_2 10 or 15 mg/kg ($P = 0.22$ and 0.46, respectively, for TC; $P = 0.2$ and 0.13, respectively, for TG). The effect of Vit K_2 administration on TC and TG levels appeared at the highest Vit K_2 dose (30 mg/kg) as there was a significant decrease of both TC and TG levels in the DM + K30 group when compared with DM rats ($P < 0.001$; Table 2).

Effects of Vit K_2 on mRNA expression levels of genes studied

A significant reduction of *osteocalcin*, *insulin 1*, *insulin 2*, *cyclin D2*, *UCP-1* and *adiponectin* mRNA expression was found in the DM group when compared with controls (15%, 50%, 40%, 27%, 19%, and 30%, respectively).

Concerning OC expression, a significant increase was found in DM + K10, DM + K15, and DM + K30 groups in a dose response when compared with DM group. We observed a dose-dependent increase of *OC* expression in the DM + K10, DM + K15, and DM + K30 by 4.45-, 5.2-, and 8.1-fold, respectively, relative to the control group (Fig. 1A).

We measured the two rat insulin genes, *Ins 1* and *Ins 2*, as well as *cyclin D2*, the gene necessary for β-cell proliferation. The DM + K10, DM + K15, and DM + K30 groups revealed significant, dose-dependent increase of *Ins 1* by 2.5-, 4.2-, and 5.1-fold, respectively ($P < 0.05$; Fig. 1B); *Ins 2* by 2.2-, 3.2-, and 4.2-fold, respectively ($P < 0.05$; Fig. 1C); and *cyclin D2* by 1.2-, 1.8-, and 2.4-fold, respectively ($P < 0.05$; Fig. 1D) in relation to controls. There was a significant upregulation of *Ins 1, Ins 2,* and *cyclin D2* gene expression in the three treated groups in a dose-dependent manner compared with DM rats.

We measured the expression of *adiponectin*, a regulator of insulin sensitivity, in white adipocytes and *UCP-1*, a molecular marker of energy expenditure, in brown adipocytes. Whereas, gene expression of *adiponectin* showed a nonsignificant increase after administration of Vit K_2 at a daily dose of 10 or 15 mg/kg, its expression was significantly increased at of 30 mg/kg daily (Fig. 1E).

Table 2
The biochemical parameters in the studied groups

	Normal control	DM	DM + K10	DM + K15	DM + K30
Body weight (g)	300 ± 19.3	480 ± 12.2*	350.2 ± 16.7*,†	334.3 ± 18.5*,†	325.6 ± 15.4*,†,‡
Fasting plasma glucose (mg/dL)	92.5 ± 2.3	346.2 ± 39.4*	153.4 ± 24.2*,†	123.6 ± 15.4*,†,‡	115.2 ± 9.8*,†,‡
Fasting insulin (μIU/mL)	19.4 ± 1.5	11.5 ± 1.2*	13.3 ± 1.4*,†	15.9 ± 2.1*,†,‡	17.4 ± 1.8†,‡
HOMA-IR	4.4 ± 0.13	9.7 ± 2.5*	5.2 ± 3.1†	4.9 ± 1.4†	4.8 ± 1.2†
HOMA-B	240.3 ± 2.4	14.7 ± 3.4*	53.6 ± 5.4*,†	96.4 ± 4.2*,†,‡	127 ± 3.5*,†,‡,§
HbA1c	5.2 ± 1.1	12.4 ± 4.2*	10.2 ± 1.3*	8.2 ± 1.7*,†,‡	5.8 ± 1.3†,‡,§
Total cholesterol (mg/dL)	65.5 ± 3.1	105.3 ± 4.4*	101.4 ± 5.6*	103.6 ± 3.2*	77.9 ± 2.3*,†,‡,§
Triacylglycerols (mg/dL)	50.7 ± 6.2	156.2 ± 3.2*	153.5 ± 3.6*	152.2 ± 5.1*	76.6 ± 3.4*,†,‡,§
Total osteocalcin (ng/mL)	8.48 ± 0.11	5.58 ± 0.1*	6.61 ± 0.43*,†	7.86 ± 0.52*,†,‡	8.46 ± 0.13†,‡,§

* $P < 0.05$ compared with control.
† $P < 0.05$ compared with DM group.
‡ $P < 0.05$ compared with DM + K10 group.
§ $P < 0.05$ compared with DM + K15.

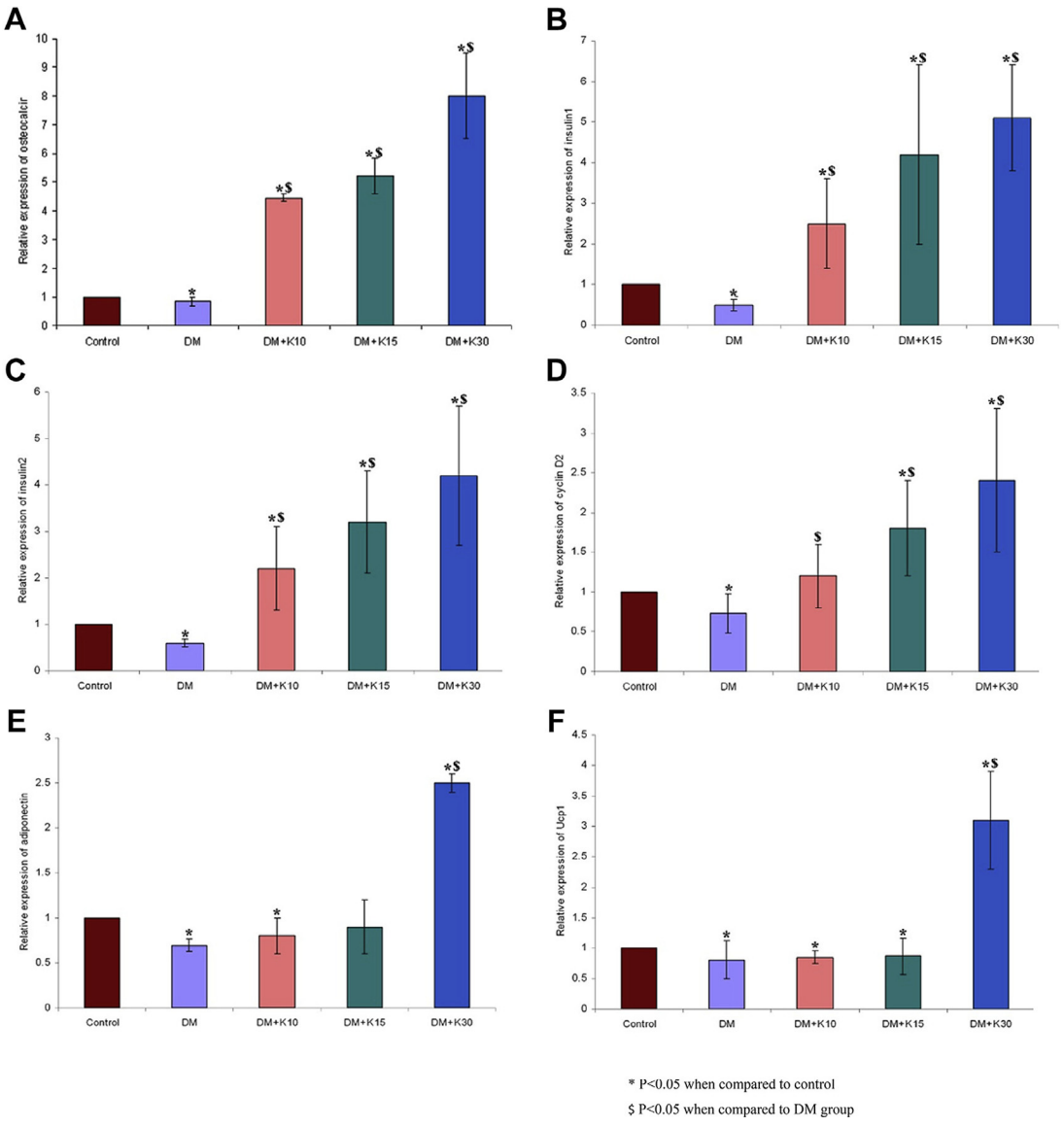

Fig. 1. Effects of vitamin K_2 on the mRNA expression levels of genes studied. (A) osteocalcin, (B) insulin 1, (C) insulin 2, (D) cyclin D2, (E) adiponectin, and (F) UCP1.

Likewise, expression of *UCP-1* was significantly increased at the highest dose (30 mg/kg) only (Fig. 1F).

Correlation between OC levels and its expression

There was a significant correlation between tOC levels and its expression in whole groups ($r = 0.89$; $P < 0.001$; Fig. 2).

Discussion

In the present study, we used menaquinone-4 (MK-4; menatetrenone), a Vit K_2 analog, which has a role in stimulation of osteoblastogenesis and inhibition of osteoclastogenesis in human bone marrow cell culture [26]. Although MK-4 showed the most potent effects in all Vit K_2 analogs [5], a lower amount was found in various foods [1]. MK-4 is essential for the γ-carboxylation of OC and increases the content of OC in cultured human osteoblasts [4].

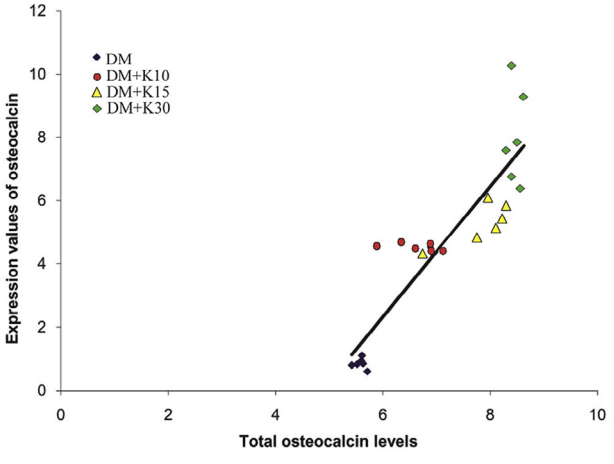

Fig. 2. Correlation between osteocalcin levels and its expression.

Evidence from animal and human studies has suggested that Vit K may be inversely associated with insulin resistance and improved glycemic status [2]. In the present study, a dose-dependent decrease was noticed after daily administration of Vit K_2 at 10, 15 and 30 mg/kg in FPG, HbA1c and HOMA-IR compared with the DM group, whereas there was a dose-dependent increase of fasting insulin and HOMA-B levels in the three treated groups. Several studies showed that higher dietary and supplemental Vit K intakes were associated with greater insulin sensitivity and better glycemic status in healthy individuals [13–16]. Beulens et al. suggested that increasing dietary Vit K intake was associated with a reduced risk for type 2 diabetes [27]. In this large cohort of 38 094 Dutch men and women, intake of both dietary phylloquinone and menaquinones was related to a reduced risk for type 2 diabetes. This association was a linear inverse for menaquinones, whereas a significant decrease for phylloquinone was shown at higher intakes [27]. In women with prediabetes, Rasekhi et al. [28] demonstrated that Vit K_1 supplementation for 4 wk did not affect insulin resistance but had good effects on the glycemic status and insulin sensitivity. Furthermore, the hypoglycemic action of Vit K_1 could have an indirect benefit by inhibiting early-onset diabetic nephropathy triggered by high blood glucose [29].

Although these studies support a potential novel role for Vit K in insulin resistance, the biological mechanisms behind the association between Vit K and glucose metabolism are unclear. Studies suggested the role of Vit K was through OC, a Vit K–dependent bone protein, which proved that it is involved in glucose metabolism. Osteocalcin is encoded by the *BGLAP* (bone γ-carboxyglutamic acid-containing protein) gene. The human *BGLAP* gene is a DNA sequence of ~1700 bp and is located at chromosome 1 q25-q31 (GenBank Acc. no. X04143.1). Pro-OC is directed to the RER membrane where the enzyme γ-glutamyl carboxylase carboxylates glutamic acid residues at the 17, 21, and 24 positions, resulting in the formation of Gla-protein in a Vit K-dependent way [30]. Animal studies proposed that ucOC may improve insulin sensitivity and increase β-cell insulin secretion [8–10]. Subsequent human studies, however, observed relations between high cOC and improved glycemic status [11,12]. This controversy gives attention that Vit K may exert its influence on glycemic status via OC through other mechanisms.

The present study suggested that Vit K_2 administration induced OC expression in a dose-dependent manner. Katsuyama et al. found that mRNAs of OC were induced after MK-7; a Vit K_2 analog, administration to the osteoblastic MC3 T3 E1 culture medium [31]. By immunocytochemical analysis, they also showed that MK-7 increased the protein levels of OC [31]. Furthermore, it has been reported that Vit K binds to the xenobiotic nuclear receptor (SXR/PXR) leading to enhanced expression of several components of the bone matrix [17,18]. So, Vit K is considered to be a transcriptional regulator of extracellular matrix–related genes [18].

In the present study, there was an increase of tOC levels after Vit K_2 administration in type 2 diabetic rats. The high OC level was associated with the decrease of insulin resistance and the increase of insulin sensitivity. Previous clinical reports have shown that higher tOC levels are correlated with better glucose homeostasis [32,33]. Among middle-aged men, elevated serum cOC and ucOC levels are associated with improved glucose tolerance and insulin sensitivity, as well as enhanced β-cell function [34]. In older men and women (only 5% with diabetes), tOC concentrations were inversely associated with FPG, fasting insulin, and HOMA-IR [11]. Recently, Liang et al. found that a low serum OC level was a risk factor for impaired glucose metabolism and subsequent type 2 diabetes [35]. In contrast, some studies did not find any correlation between ucOC or tOC and glucose levels in patients with normal glucose tolerance [13,16,36]. These studies attest to the fact that the effects of OC on β-cell function have been observed only among individuals with prediabetes and diabetes or among individuals with an abnormal glucose metabolism.

In the present study, we found a decrease of OC expression and its protein levels in the diabetic group. Lower OC levels have been widely found in patients with diabetes [32,37,38]. However, it remains to be clarified if low OC concentrations in type 2 diabetes illustrate a cause of or a result of hyperglycemia, or both. A mischievous effect of hyperglycemia on the bone has been found [39], whereas a protective effect has been attributed to high concentrations of OC [11].

In the present study, we embarked on OC signaling pathways of regulation of glucose metabolism. We found increased expression of the *Ins 1* and *Ins 2* insulin genes and *cyclin D2* gene associated with increased *OC* gene expression and high serum OC levels. Gene expression analyses of islets or cultured β-cell lines treated with OC have shown that this hormone directly increases the expression of not only the *insulin* genes but also cyclin-dependent kinase 4 (*Cdk4*), *cyclin D1,* and *cyclin D2* [5–7]. These results suggested that OC could directly regulate β-cell proliferation.

Osteocalcin was suggested to function as a hormone in energy metabolism, regulating insulin sensitivity through an effect on adiponectin [6]. In the present study, we found an increase of expression of *UCP-1*, a molecular marker of energy expenditure in brown adipocytes and expression of adiponectin gene in white adipocytes associated with higher levels of OC. Adiponectin, which decreases as fat mass increases, is known to be an important factor in the regulation of insulin sensitivity. OC-knockout mice showed an increased fat mass, high glucose and TG levels, and a decrease in secretion of insulin and adiponectin [8], whereas administration of ucOC increased the insulin and adiponectin secretion and stimulated the glucose and lipid catabolism [6].

In attempting to estimate the doses of Vit K_2 that are able to affect glucose and energy metabolisms in type 2 diabetes, we gave the diabetic-induced rats three different doses [21–23]. We found a dose-dependent decrease in FPG, HbA1c, and HOMA-IR after daily administration of Vit K_2 at 10, 15, and 30 mg/kg when compared with DM group. Also, a dose-dependent increase in fasting insulin and HOMA-B values was indicated. There was a dose-response increase of OC and OC target genes (*Ins 1, Ins 2,* and *cyclin D*) expression. In vitro and in vivo studies showed that β-cell proliferation and *insulin* expression are significantly affected by relatively low concentrations of OC (6–60 pM), whereas higher concentrations (0.6–6 nM) did not [6]; a result not accordance with ours. In the present study, we found that gene expression of adiponectin showed a nonsignificant increase after administration of Vit K_2 at a dose 10 of 15 mg/kg; its expression was significantly increased at a daily dose of 30 mg/kg (highest level of OC). Likewise, expression of *UCP-1* was significantly increased at the highest dose (30 mg/kg) only. Also, the effect of Vit K_2 administration on TC and TG levels appeared at the highest Vit K_2 dose. In accordance with the present study, Ferron et al. found that adiponectin, *Pgc1* α and *Ucp1* (markers of energy expenditure), *Acyl-CoA oxidase*, *Ucp2*, and *Ppar* α (adiponectin target genes) expression was maximally enhanced by higher concentrations of OC [6].

Sakamoto et al. [40] administered menaquinone-4 (90 mg/d for 1 wk) in young male volunteers and examined the insulin and

glucose levels during 75 g oral glucose loading before and after Vit K intake. Interestingly, the volunteers with higher serum descarboxyprothrombin levels (i.e., lower Vit K) showed the largest decrease in insulin secretion. Another study found that Vit K supplementation for 36 mo at doses obtainable in the diet may reduce progression of insulin resistance in older men [13]. One of the limitations of the present study was that the STZ-induced DM model was likely different in underlying mechanisms, at least in part, from the prevailing type 2 DM as a lifestyle-related disease.

Conclusion

Vit K_2 administration could improve glycemic status in type 2 diabetic rats by induction of OC gene expression. Osteocalcin could increase β-cell proliferation, energy expenditure, and adiponectin expression. We found also that different concentrations of Vit K_2 were required to affect glucose metabolism and insulin sensitivity. This study starts addressing the therapeutic potential of this new player in the regulation of glucose homeostasis in patients with diabetes.

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