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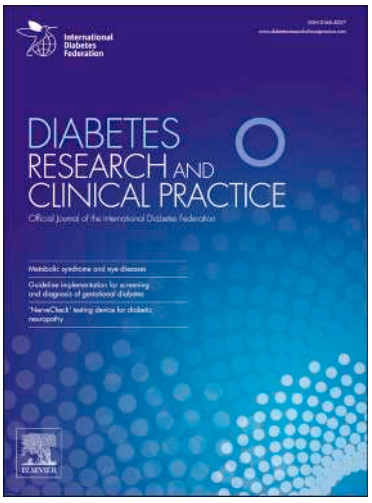

Review

Effect of Vitamin K2 on Type 2 Diabetes Mellitus: A Review

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PII: S0168-8227(17)31256-1
DOI: https://doi.org/10.1016/j.diabres.2017.11.020
Reference: DIAB 7140

To appear in: *Diabetes Research and Clinical Practice*

Received Date: 6 August 2017
Revised Date: 31 October 2017
Accepted Date: 16 November 2017

Please cite this article as: Y. Li, J. peng Chen, L. Duan, S. Li, Effect of Vitamin K2 on Type 2 Diabetes Mellitus: A Review, *Diabetes Research and Clinical Practice* (2017), doi: https://doi.org/10.1016/j.diabres.2017.11.020

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Title Effect of Vitamin K2 on Type 2 Diabetes Mellitus: A Review

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Abstract

Type 2 diabetes mellitus (T2DM) continue to be a major public health problem around the world that frequently presents with microvascular and macrovascular complications. Individuals with T2DM are not only suffering from significant emotional and physical misery, but also at increased risk of dying from severe complications. In recent years, evidence from prospective observational studies and clinical trials has shown T2DM risk reduction with vitamin K2 supplementation. We thus did an overview of currently available studies to assess the effect of vitamin K2 supplementation on insulin sensitivity, glycaemic control and reviewed the underlying mechanisms. We proposed that vitamin K2 improved insulin sensitivity through involvement of vitamin K-dependent-protein osteocalcin, anti-inflammatory properties, and lipid-lowering effects. Vitamin K2 had a better effect than vitamin K1 on T2DM. The interpretation of this review will increase comprehension of the development of a therapeutic strategy to prevent and treat T2DM.

Key Words： Vitamin K2; Diabetes Mellitus, Type 2; Insulin Resistance; Osteocalcin; Adiponectin; Inflammation

Introduction

T2DM manifests when pancreatic β-cells fail to compensate for chronic elevated blood glucose (hyperglycemia) that occurs when glucose uptake in the insulin-sensitive tissues become imbalanced during insulin resistance. Insulin resistance, a state that precedes T2DM, is one of the major sign of pathogenesis

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and etiology of T2DM. It is becoming increasingly obvious that chronic, low-grade systemic inflammation attends obesity, and insulin resistance leads to the progression from obesity to T2DM. T2DM frequently presents with microvascular and macrovascular complications, lead to long-term damage, malfunction and failure of various organs and systems, especially the eyes, kidneys, nervous and cardiovascular systems. Importantly, it has been considered as a strong, independent risk factor for cardiovascular diseases that accounts for approximately 70% of all mortality in patients with diabetes. If patients fail to maintain normal blood glucose levels, the long-term complications will impose significant emotional or physical burdens on patients, family and society.

In recent years, evidence from prospective observational studies and clinical trials has shown T2DM risk reduction with vitamin K supplementation. Vitamins are vital micronutrients that the organism cannot synthesize sufficient quantity and obtained mainly from diet. Henrik Dam, who later shared the 1943 Nobel Prize in medicine with Edward Doisy for their work on vitamin K, in 1935, discovered a fat soluble factor with similar physical properties to vitamin E but different physiological clotting function from any known vitamin. Dam called this new factor "the anti-hemorrhagic vitamin" which finally got the name "vitamin K" based on the Scandinavian and German spelling of ''Koagulations''[1]. Vitamin K also functions as a cofactor for γ-glutamyl carboxylase which is essential for the conversion of glutamate (Glu) residues to

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γ-carboxyglutamate (Gla). Two vitamin K- dependent-proteins, osteocalcin and matrix-Gla protein, present in skeletal and vascular system, and vitamin K has been reported plays a role in cardiovascular system and skeletal health[2].

Vitamin K exists mainly in two biologically active forms: vitamin K1 (also known as phylloquinone) and vitamin K2 (also known as menaquinone). Vitamin K1 is naturally produced by plants and most abundant in green leafy vegetables[3]. Vitamin K2 is predominantly found in meat, eggs, curd, cheese, and fermented soybeans (natto). In addition, bacteria in human intestine also synthesize menaquinone. Menaquinones are abbreviated as MK-n where M represents menaquinone, K represents vitamin K, and n stands for the number of isoprenoid side chain length[4].

The importance of vitamin K2 for osteoporosis and cardiovascular disease is well-recongnized[5, 6], and there are some suggestions of a role for vitamin K2 in improving insulin sensitivity and reducing T2DM risk[7, 8]. Here we provided an overview of currently available studies to assess the effect of vitamin K2 supplementation on insulin sensitivity, glycaemic control and review the underlying mechanisms. We proposed that vitamin K2 improved insulin sensitivity through involvement of vitamin K-dependent-protein osteocalcin, anti-inflammatory properties, and lipid-lowering effects. Vitamin K2 had a better effect than vitamin K1 on T2DM. The interpretation of this review will promote the development of a potential therapeutic strategy to prevent and treat T2DM.

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1. Vitamin K2 reduces the risk of T2DM:

Beulens et al. (2010) examined the associations of dietary phylloquinone and menaquinone intakes with the risk of T2DM in a large samples of 38,094 Dutch men and women (20–70 y) in prospective cohort study. Dietary phylloquinone and menaquinone intakes were analyzed by using a food-frequency questionnaire (FFQ). It had been observed that menaquinone intake tended to be inversely associated with risk of T2DM with an Hazard Ratio of 0.95 for each 10-µg increment (P=0.060). In the final multivariate model, a linear, inverse association was observed with an HR of 0.93 for each 10-µg increment of menaquinones intake (P=0.038). This finding showed 7% T2DM risk reduction with each 10-µg increment of vitamin K2 intake[8].

A study by Choi et al. (2011) also reported a relationship between insulin sensitivity and vitamin K2 supplementation (MK-4; 30 mg; 4 wk) among healthy young men (n=42). Vitamin K2 supplementation was found to be associated with increased insulin sensitivity index (P= 0.01) and disposition index ($P < 0.01$), but these indices were not affected by placebo treatment[9].

In another study, Zatollah et al. (2016) examined the effect of vitamin D, K and Ca co-supplementation on carotid intima-media thickness (CIMT) and metabolic status among overweight diabetic patients with CHD. They conducted a randomised, double-blind, placebo-controlled trial among sixty-six diabetic patients who were randomly allocated into two treatment groups. Results suggested that changes in insulin concentrations (P=0.01), HOMA-IR (P=0.01),

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β-cell function (P=0.01) in supplemented patients were significantly different from those in the placebo group[10]. (Table. 1)

The effect of vitamin K supplementation on insulin sensitivity and glycemic status has been reviewed by Manna et al. (2016). They integrated currently available evidences and proposed that both vitamin K1 and vitamin K2 supplementation were beneficial to the reduced risk of T2DM[11]. This also suggests a beneficial role of vitamin K2 on insulin sensitivity and glucose metabolism.

2. Mechanisms underlying the effect of vitamin K2 on T2DM

2.1 Vitamin K2 improves insulin sensitivity via osteocalcin metabolism

2.1.1 Osteocalcin is a vitamin K- dependent protein

Vitamin k shares the common structure, 2-methyl-1,4-napthoquinone, functioning as a cofactor for the enzyme, γ-glutamate carboxylase (GGCX) which is essential for vitamin K-dependent-protein to convert glumate to γ-carboxyglutamic (Gla) residues. Osteocalcin is a kind of vitamin K-dependent –protein and synthesized by osteoblasts. It contains a propeptide recognition site that is essential for binding to GGCX and undergoes an unusual post-translational modification. After carboxylation, the propeptide is removed and the mature osteocalcin is secreted[12]. The γ-carboxyglutamic residues in osteocalcin are invovled in the regulation of size and shape of bone mineral and bone metabolism. Excessive vitamin K intake results in increased Gla-OC, suboptimal vitamin K intake results in increased Glu-OC.

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There are three vitamin K-dependent γ-carboxyglutamic acid sites in osteocalcin molecule. In most species, all three sites are fully carboxylated. While in human, osteocalcin in bone and serum is not completely carboxylated (undercarboxylated osteocalcin). Analysis of osteocalcin from 20 human bone samples found carboxylation to be mean±SD 67±14% at Glu17, 88±9% at Glu21, and 93±4% at Glu24[13]. Circulating osteocalcin is similarly incompletely carboxylated. Estimates of the percentage of undercarboxylated osteocalcin suggested that up to 50% of osteocalcin was undercarboxylated in serum of normal individuals. Thus there should be three forms of osteocalcin mentioned in studies about osteocalcin, including carboxylated osteocalcin (cOC), undercarboxylated osteocalcin and uncarboxylated osteocalcin. But most studies did not accurately distinguish the latter two forms of OC, as there were some limitations in the measurement of them. Generally high serum undercarboxylated osteocalcin or uncarboxylated osteocalcin reflects a low, and high carboxylated osteocalcin reflects a high vitamin K status[14].

2.1.2 Osteocalcin favors β-cell proliferation, insulin secretion and sensitivity

Lee NK et al. (2007) investigated endocrine regulation of energy metabolism by the skeleton. They demonstrated that osteocalcin favors β-cell proliferation, insulin secretion and sensitivity by stimulating β-cells expressing *CyclinD1* and *Insulin* and adipocytes expressing *Adiponectin*[15]. By taking advantage of osteoblast paucity of cell-specific gene expression, they generated mutant mouse strains lacking genes encoding signaling molecules expressed in

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osteoblasts. Through this effort they inactivated *Esp*, a gene that encodes a protein tyrosine phosphatase OST-PTP. Mice lacking *Esp* in osteoblasts remarkably displayed an increase in β-cell proliferation, insulin secretion and sensitivity; but all these phenotypes were corrected by deleting one allele of *Osteocalcin*; moreover, mutant mice with *Osteocalcin* -/- were glucose intolerant and fat. Their genetic assays showed that osteocalcin favored pancreatic β-cells proliferation, *Insulin* expression, and *Adiponectin* expression in adipocytes. [15].

Later, Pittas et al. (2009) examined the associations of serum osteocalcin concentration and measures of dysmetabolic phenotype using data from a clinical trial among adults (n=380, 71 y, 5% with diabetes)[16]. Saleem et al. (2010) also examined the associations of serum osteocalcin and measures of insulin resistance, circulating adipokine levels, and the presence of metabolic syndrome (MetSyn) among 1284 blacks (64+/-9 y) and 1209 whites (59+/-10 y)[17]. Two clinical trials reached consistent conclusions that serum osteocalcin was involved in the regulation of glucose metabolism. Furthermore, it was observed by Gravenstein et al. (2011) that bone and glucose metabolism were probably connected through a complex pathway including leptin, osteocalcin, and adiponectin in a cross-sectional study[18]. (Table. 2(A))

2.1.3 There is an inconsistency in the form of osteocalcin involved in glucose metabolism

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Lee et al. reported the role of uncarboxylated osteocalcin in regulating glucose metabolism using genetically modified mice. Uncarboxylated osteocalcin increased β-cell proliferation, insulin secretion, and regulated insulin sensitivity via stimulating the expression of adiponectin[15]. However vitamin K supplementation (vitamin K1 and K2), which caused a decrease in the levels of uncarboxyleted osteocalcin and an increase of carboxylated osteocalcin, had been reported to reduce insulin resistance among patients at high risk of T2DM. What's more, various cross-sectional and longitudinal studies showed that serum carboxylated osteocalcin were inversely related to the measures of glucose metabolism, such as fasting glucose, HOMA-IR, and so on.

Choi et al. (2011) demonstrated the beneficial role of vitamin K2 supplementation in increasing insulin sensitivity in healthy young men. Intravenous glucose tolerance test was performed to determine insulin sensitivity index (S_i), ucOC and cOC levels were measured before and after treatment. Results showed that vitamin K2 supplementation significantly increased S_i (4.4 vs. 6.6; P=0.01), these indices were not affected by placebo treatment. Treatment with vitamin K2 decreased ucOC (0.9 vs. 0.4 ng/ml; P=0.02), and increased cOC (9.6 vs. 16.0 ng/ml; P=0.01). This suggests a beneficial role of vitamin K2 in increasing insulin sensitivity via the effect of carboxylated osteocalcin[9].

Shea et al. (2009) examined associations of circulating forms of osteocalcin and insulin resistance in older men and women in cross-sectional and

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longitudinal studies. They examined associations of serum total osteocalcin, cOC and ucOC and insulin resistance in nondiabetic men and women (n=348) in cross-sectional study by using HOMA-IR. They also examined associations of each form of osteocalcin at baseline and 3-y change in HOMA-IR in adults (n=162). It had been observed that subjects in the lowest tertiles of carboxylated osteocalcin had higher baseline HOMA-IR (P=0.02). Furthermore, the level of carboxylated osteocalcin at baseline was inversely related to a 3-year change in HOMA-IR (P=0.002)[19].

Hwang et al. also found the effect of carboxylated osteocalcin on insulin sensitivity among middle-aged male subjects (n=199, mean at 47 y) in a cross-sectional study. Both uncarboxylated and carboxylated osteocalcin plasma levels, OGTT, HOMA-IR and other metabolic parameters such as BMI were measured. Results showed that the upper cOC tertile was inversely associated with plasma glucose level and more closely related to increased insulin sensitivity ($P<0.05$)[20]. In addition, they found body weight and BMI were significantly lower in the upper tertile of cOC. This finding was consistent with the result of Knapen et al, who investigated the effect of vitamin K treatment on adiponectin, body weight and BMI in archived samples from 42 young men and women and 164 postmenopausal women. They also found subjects with higher cOC were leaner and had less body fat compared to those with lower cOC[21].

The effect of osteocalcin on insulin sensitivity had also been investigated by Pollock et al. (2011) among overweight prupubertal children with normal

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glucose levels (n=99) and prediabetes (n=41). OGTT was used to identify prediabetes and measures of insulin sensitivity (Matsuda index). Results demonstrated that both in the normal-glucose and prediabetes group, cOC was positively associated with insulin sensitivity (β=0.26, 0.47, respectively, both P<0.02)[22]. (Table. 2(B))

2.1.4 The thinking about the possible reasons for the inconsistency

The possible reasons for the inconsistency could be speculated as follows：Firstly, there are genetic differences between mice and human, the human osteocalcin gene is a single copy gene located at the distal 1q, while mice have a cluster of three osteocalcin genes in a 23 kb span oriented[23]. In addition, OC gene is upregulated by vitamin D in human, while downregulated in mice. Moreover, it has been proposed that *Esp* is a pseudogene in human, but two close homologs were expressed in osteoblasts may replace its function[24]. Secondly, serum OC level shows diurnal variation in human and increases during ageing, growth, skeletal maturation and menopause[5]. Thirdly, the undercarboxylated OC in human circulation would be the cosequence of two processes: suboptimal vitamin K intake and decarboxylation during osteoclast resorption. However, most of the human studies do not take into account vitamin K intake and the independent measures of bone resorption and formation[14]. Finally, there are some limitations in the measurement of the different forms of osteocalcin, including cOC, undercarboxylated osteocalcin and uncarboxylated

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OC, thus the interpretation may bia the results[25]. Therefore, accurate studies are needed to define the forms of osteocalcin invovled in glucose metabolism.

2.1.5 Dose adiponectin play a role in the effect of vitamin K2 supplementation on increased insulin sensitivity?

The mechanisms underlying vitamin K2 increased insulin sensitivity via osteocalcin metabolism were unclear, but there are speculations that osteocalcin regulates insulin sensitivity through the effect of adiponectin in human. Lee at al. demonstrated that osteocalcin regulated *Adiponectin* expression independently of its effct on insulin secretion. This finding supported the speculation that osteocalcin regulated insulin sensitivity independently of its effect on insulin secretion, and this regulation occurred partly through adiponectin[15]. What's more, Zhang yong et al. (2017) demonstrated that vitamin K2 (MK-7) intervention was associated with increased serum adiponectin level[26].

Plasma adiponectin level has been shown inversely associated with BMI in animals and human studies[27]. Lower plasma adiponectin level was found in obese subjects compared to lean subjects. Furthermore, adiponectin level was down-regulated in obesity and positively associated with insulin sensitivity[28]. And negative correlations between adiponectin and IR have been shown repeatedly[29, 30]. That adiponectin enhances insulin sensitivity appears to be through increased fatty acid oxidation and inhibition of hepatic glucose production[31]. The adiponectin receptor 1 (AdipoR1) was found to be predominantly expressed in skeletal muscle and showed a high-affinity for

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globular adiponectin. It activated 5'-AMP-activated protein kinase (AMPK), peroxisome proliferator-activated receptors α(PPAR-α) and p38 mitogen-activated protein kinase (MAPK), then increased glucose uptake and fatty acid oxidation. While adiponectin receptor 2 (AdipoR2) was most abundant in the liver and seemed to be predominantly mediating the effect of full-length adiponectin. It can also activite AMPK and p38 MAPK, and decreased expression of hepatic gluconeogenic enzymes, then inhibited glucose production[32]. Taken together, vitamin K2 improves insulin sensitivity via osteocalcin metabolism, and it's resonably to speculate that adiponectin play a role in this pathway. (Fig. 1)

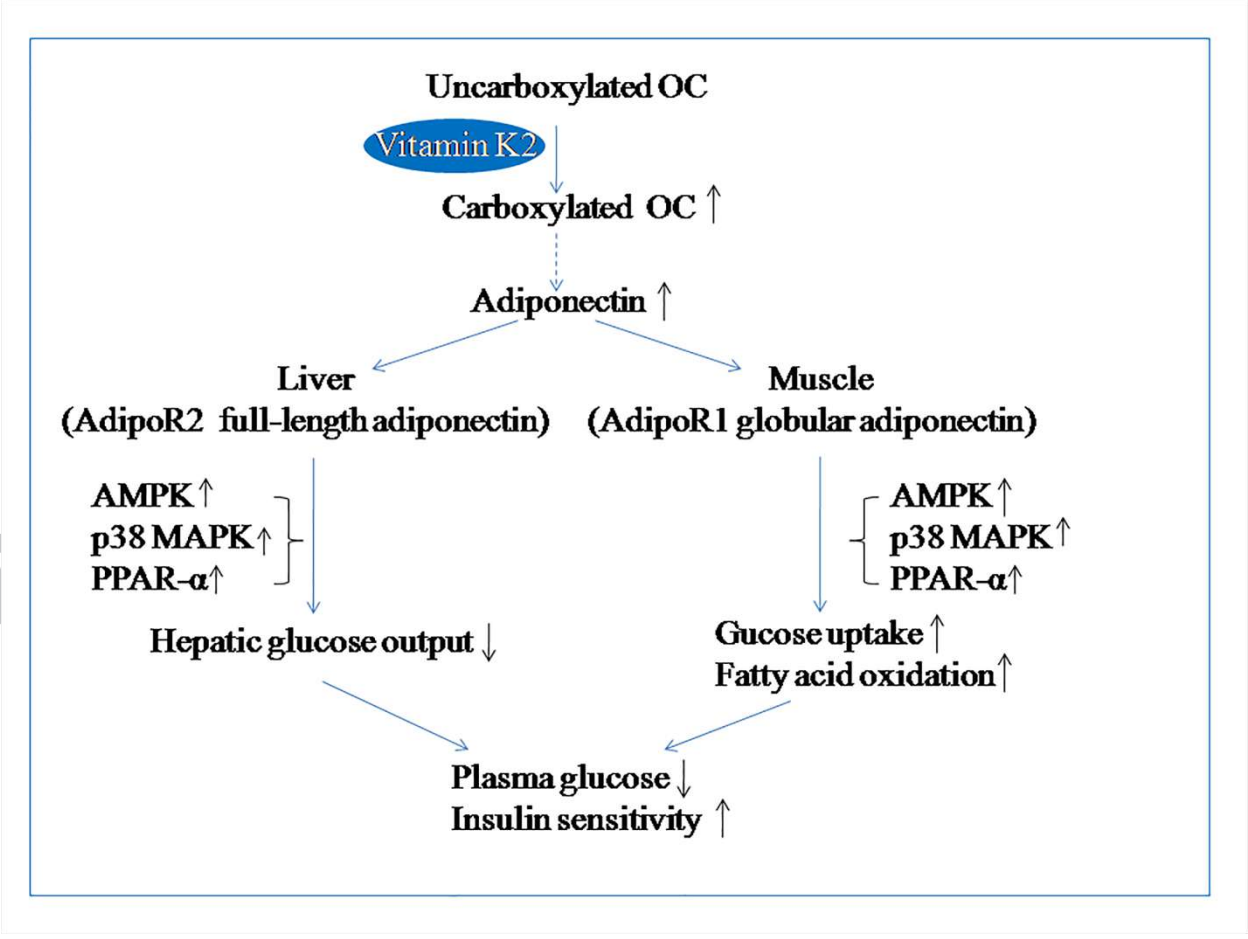

Fig. 1 Vitamin K2 improves insulin sensitivity via osteocalcin metabolism, and

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adiponectin might play a role in this pathway. Vitamin k2 supplementation increased carboxylated osteocalcin, and it was speculated that osteocalcin influenced insulin sensitivity through the effect of adiponectin. In the liver, AdipoR2 predominantly mediated the effect of full-length adiponectin and increased phosphorylation of AMPK, p38 MAPK, and PPAR-α. Decreased expression of hepatic gluconeogenic enzymes inhibited glucose production and decreased plasma glucose. In skeletal muscle, AdipoR1 mediated the effect of globular adiponectin and increased phosphorylation of AMPK, p38 MAPK and PPAR-α. This increased glucose uptake and fatty acid oxidation, finally increased insulin sensitivity. OC, osteocalcin; AdipoR1, adiponectin receptor 1; AdipoR2, adiponectin receptor 2; AMPK, 5'-AMP-activated protein kinase; p38 MAPK, p38 mitogen-activated protein kinase; PPAR-α, peroxisome proliferator-activated receptors α.

2.2 Vitamin K2 improves insulin sensitivity via anti-inflammatory effect

2.2.1 Inflammatory responses play a crucial role in the pathogenesis and development of insulin resistance

Insulin resistance (IR) is one of the main hallmark for pathogenesis and etiology of T2DM, and one of the main causative factors for the etiology of T2DM. Overnutrition is a major causative factor that contributes to induce the state of low-grade inflammation, and IR is mainly induced by various pro-inflammatory mediators such as interleukin-1β (IL-1β), interleukin-6 (IL-6) [33], tumor necrosis factor-α (TNF-α)[34], numerious chemokines and adipocytokines, glucolipotoxicity, and various transcriptional and metabolic pathways[35].

Rehman et al. (2016) comprehensively collected data from the searched scientific literatures and experimental evidences. They found that inflammatory responses activated the production of various pro-inflammatory mediators

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notably cytokines, chemokines and adipocytokines. They described how pro-inflammatory mediators, transcriptional mediated molecular and metabolic pathways were involved in the pathogenesis of IR[36].

IL-6 prevents non-oxidative glucose metabolism and suppresses the lipoprotein lipase which consecutively increases the plasma levels of triglycerides[37]. Besides, IL-6 activates the suppressor of cytokine signaling (SOCS) proteins[38], which may block activation of insulin transcriptional factor. Signal transducer and activator of transcription 5B (STAT5B) is a protein has unique ability to act as signal transducer. It binds with phosphotyrosine 960 of the insulin receptor, then potentiates the tyrosine kinase and activates insulin transcription factor. SCOS protein significantly competes with STAT5B that supresses the tyrosine kinase and blocks the activation of insulin transcripton factor[38]. (Fig.2)

TNF-α binding with its receptor TNF-R1 results in the activation of two major transcriptional factors, nuclear factor kappa-B (NF-κB) and Jun NH2-terminal kinase (JNK)[39]. In the TNF signal transduction pathway, TNF-α binding to TNF-R1 results in the formation of a TNF-R1 receptor complex including important adaptor proteins TRADD, TRAF2, RIP and FADD. These adaptor proteins recruit additional key pathway-specific enzymes (for example, IKK complex) to the TNF-R1 complex, where they are activated and initiate NF-κB and JNK activation. The activation of JNK and IKK in the payhway potentiated serione/theronine kinase and resulted in increased

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phosphorylation of IRS on serine, which inhibited phosphorylation of IRS on tyrosine, thus interfered the insulin signaling pathway. NF-κB is a transcriptional mediated factor that regulates various inflammation responses, it targets several genes to potentiate the release of numerous pro-inflammatory mediators such as IL-1β, IL-6 and TNF-α. These cytokines enter into the blood stream and induce IR in tissues. (Fig.2) Besides, TNF-α was found can down-regulate adiponectin mRNA levels in 3T3-L1 cells[40, 41], which is positively associated with insulin sensitivity.

2.2.2 Vitamin K2 suppresses inflammatory responses via the inactivation of the NF-κB signalling pathway.

Ohsaki et al. (2010) demonstrated that vitamin K (MK-4) suppressed lipopolysaccharide-induced expression of inflammatory cytokines by inhibiting of the activition of NF-κB via the repression of IKKα/β phosphorylation. Human monocytic THP-1 and mouse RAW264.7 cells were incubated in a culture medium of vitamin K analogues, and stimulated by LPS (1 μg/mL). Then RNA was isolated from these cells 3 h after LPS stimulation. RT-PCR was used for amplification, Western blot analysis was used for protein detection. Results showed firstly, MK-4 suppressed the increased IL-6 expression in LPS-treated human THP-1 cells ($P<0.05$), and suppressed the increased mRNA levels of IL-6, TNFα, and IL-1β in LPS-treated mouse RAW264.7 cells. Secondly, they found vitamin K analogues, such as vitamin K1, MK-3, MK-4, MK-7 also suppressed the LPS-induced IL-6 expression in human THP-1 cells ($P<0.05$).

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The common 2-Methyl-1,4-naphthoquinone ring structure contributed to express the anti-inflammatory effect. In addition, warfarin did not affect the IL-6 mRNA levels decreased by MK-4 treatment which indicated effects of vitamin K were independent of its Gla formation activity. Thirdly, LPS-induced NFκB activation was inhibited by pretreatment with MK-4 in THP-1 cells. LPS stimulation triggered a phosphorylation cascade of proteins that resulted in the activation of NFκB. They measured the phosphorylation levels of cascade proteins in the NFκB activation pathway to determine the responsible molecule inhibited by MK-4. They found that phosphorylation of IKKα/β and NFκB p65 was significantly reduced, this indicated the inhibition of NFκB activity by MK-4 is result of the inhibition of activation of IKKα/β[42].

The suppression of IL-6 expression by vitamin K has been previously investigated by other researchers. Reddi et al. (1995) reported the LPS-induced IL-6 secretion was suppressed by vitamin K in human fibroblasts. The compunds examined in their study included phylloquinone (VK1), menaquinone-4 (VK2), menadione (VK3). All of these compounds were capable of inhibiting IL-6 production with the rank order K3>K2>K1[43].Vitamin K analogues suppressed the LPS-induced IL-6 expression in human cells via inhibition of NFκB pathway activity.(Fig. 2) Thus it's plausible that vitamin K2 improves insulin resistance by suppressing inflammatory responses via the repression of the NF-κB activition. Anti-inflammatory treatment has been proposed as a treatment strategy for IR, thereby vitamin K2 is a potential

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therapeutic strategy for T2DM in the future.

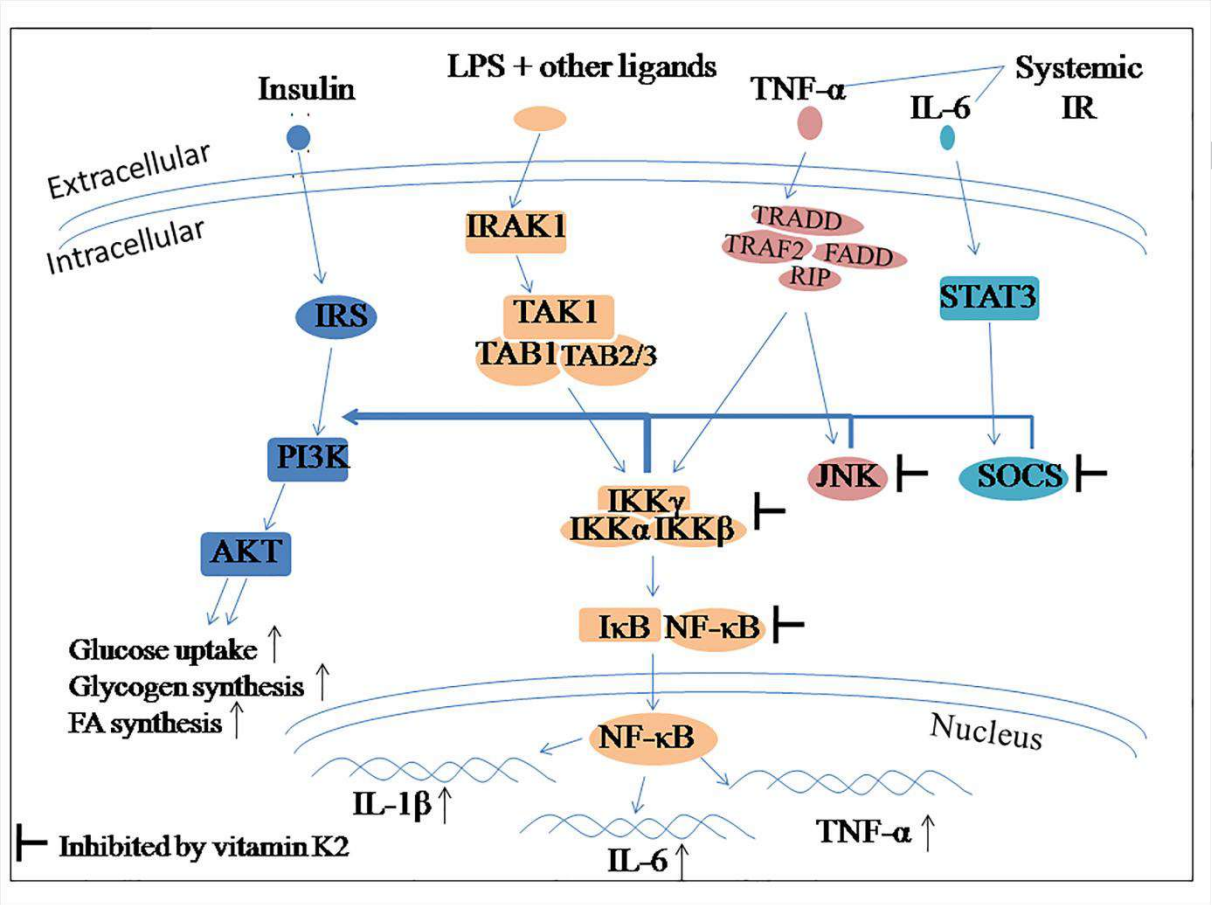

Fig. 2 Vitamin K2 improves insulin resistance by suppresseing inflammatory responses via the inactivation of the NF-κB signaling pathway.

1. The activation of SOCS protein, JNK and IKK complex inhibits phosphorylation of IRS on tyrosine. IL-6 activates the SOCS proteins, which competes with signal transducer and activator of transcription 5B (STAT5B), then supresses the tyrosine kinase and phosphorylation of IRS on tyrosine; TNF-α binds to TNF-R1 and results in the formation of a TNF-R1 receptor complex, then activates the IKK complex and initiates NF-κB and JNK activation. The activation of JNK and IKK increases phosphorylation of IRS on serine and inhibits phosphorylation of IRS on tyrosine, thus interferes with insulin signaling pathway and induces IR.

2. Vitamin K2 suppresses inflammatory responses via the inactivation of the NF-κB signaling pathway. Vitamin K2 reduced phosphorylation of IKKα/β, then inhibits NFκB activation and suppresses the expression of IL-6, IL-1β, TNF-α. Reduction of these pro-inflammatory mediators decreases activation of SOCS protein, JNK and IKK complex again. Thus vitamin

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K2 improves insulin resistance by suppressing inflammatory responses.
IRS, insulin receptor substate; PI3K, phophatidylinostitol 3-kinase; AKT, protein kinase B; IκB, inhibitor of κB; IKK, IκB kinase; NF-κB, nuclear factor kappa-B; TRADD, TRAF2, RIP and FADD, TNF-R1 receptor complex; JNK, Jun NH2-terminal kinase; SOCS, suppressor of cytokine signaling.

2.3 Vitamin K2 improves insulin resistance via Lipid-lowering efficacy

2.3.1 Obesity and IR

Obesity is associated with increased deposition of lipids in non-adipose tissues like skeletal muscle, liver, and pancreatic β-cells. These lipids continuously derive long-chain fatty acyl Co (LC-CoA) and other metabolites that act as signaling molecules on protein kinase activities, ion channel, gene expression, and protein acylation. In pancreatic β-cells, short-term exposure to fatty acids or LC-CoA activates PKC and directly stimulates insulin exocytosis, while long term excess of FFA leads to blunted glucose-stimulated insulin secretion. In skeletal muscle, on the one hand, excessive fatty acids supply reduces hexokinase activity and leads to decreased glucose oxidation. The accumulation of glucose-6-phosphate in turn decreases insulin-stimulated glucose uptake. On the other hand, accumulated intramuscular triglycerides gives rise to LC-CoA and their derivatives, which are activators of protein kinase C (PKC) isoenzymes. The increase in LC-CoA activates PKC isoforms and phosphorylates insulin receptor substrate (IRS-1) on serine. While the serine phosphorylation of IRS-1 prevents phosphorylation of IRS-1 on tyrosine and further binding and activation of PI3 kinase. This interrupts the insulin signaling

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pathway and leads to impaired insulin sensitivity in the insulin-sensitivity tissues[44].

2.3.2 Vitamin K2 supplementation decreases fat accumulation and serum triglycerides

Nagasawa et al. (1998) found vitamin K2 reduced total cholesterol concentrations by administering vitamin K2 for 4 to 236 months to chronic renal failure patients treated with continuous ambulatory peritoneal dialysis [45]. Later, Kawashima et al. (1999) demonstrated that the pharmacological dose of vitamin K2 reduced the total-cholesterol, lipid peroxidation in plasma by treating 24 hypercholesterolemic rabbits with vitamin K2 in daily doses of 1, 10 and 100 mg/kg with a 0.5% cholesterol diet for 10 weeks[46].

Sogabe et al. (2011) reported long-term addition of menaquinone-4 (MK-4) significantly decreased the total fat accumulation and serum triglycerides. They conducted a long-term addition of phylloquinone or MK-4 (600mg/kg diet, 3 mo) to a control diet in 23 female SD rats. Body composition and serum parameters were measured. Results showed MK-4 significantly decreased the total fat accumulation ($p<0.05$) and serum triglycerides were reduced by 29% in the MK-4 group compared to the control group[47].

The association of the dietary intake and/or status of menaquinone and metabolic syndrome (MetS) and it's compnents has also been examined by Dam et al. (2015). They demonstrated the relationship of menaquinone intake with MetS was mainly driven by lower triacylglycerol concentrations and lower waist

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circumference. Baseline menaquinone intakes were measured with a validated FFQ and the mean menaquinone intake was 31.1±12.5μg/d. Results showed higher menaquinone intake was associated with a lower prevalence of MetS (Ptrend=0.08). Longitudinal analysis also demonstrated the significant association of menaquinone intake with the lower prevalence of MetS (Ptrend=0.01)[48].

Lipid metabolism disorders induced hyperlipidemia and ectopic fat deposition has been considered as the main mechanism of obesity induced IR. Vitamin K2 supplementation significantly decreases the fat accumulation and serum triglycerides, therefore it's plausible that vitamin K2 improves insulin resistance via the lipid-lowering efficacy. Based on the emerging new insights into the regulation of lipid metabolism, vitamin K2 supplementation is one of the potentially novel therapeutic strategies to treat IR and reduce the risk of T2DM.

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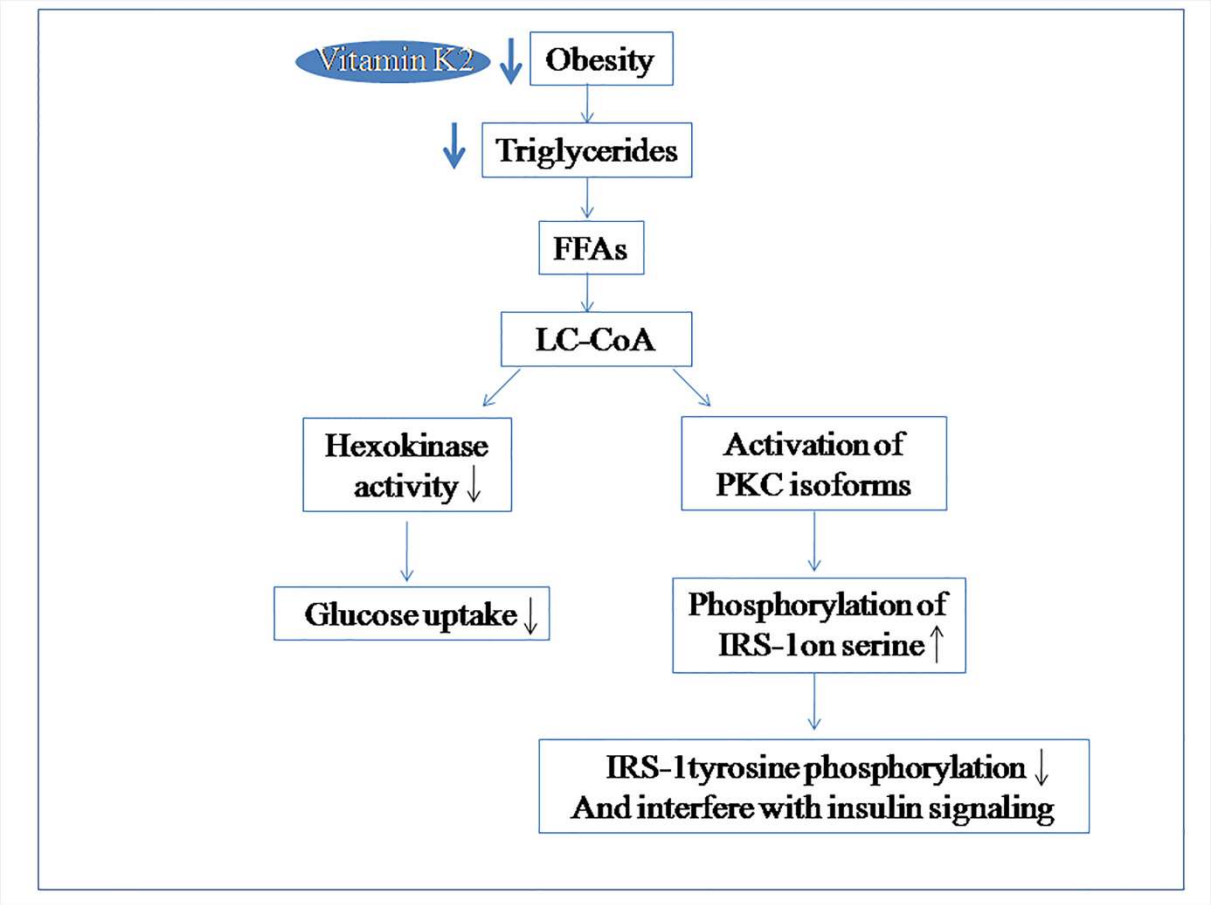

Fig. 3 Vitamin K2 improves insulin resistence via Lipid-lowering efficacy. Obesity increases deposition of lipids in non-adipose tissues like skeletal muscle, liver, and pancreatic β-cells. These lipids continuously derive long-chain fatty acyl CoA (LC-CoA) and other metabolites that act as signaling molecules on protein kinases activities. In skeletal muscle, on the one hand, excessive fatty acids supply reduces hexokinase activity, and leads to accumulation of glucose-6-phosphate, which in turn decreases insulin-stimulated glucose uptake. On the other hand, the increase in LC-CoA activates PKC isoforms and phosphorylates IRS-1 on serine. While the serine phosphorylation of IRS-1 prevents phosphorylation of IRS-1 on tyrosine. This interrupts insulin signaling pathway and leads to decreased insulin sensitivity in the skeletal muscle as well as other insulin-sensitivity tissues. However, sveral clinical studies have shown that vitamin K2 supplementation decreases total cholesterol concentrations, fat accumulation and serum triglycerides. Thus it's plausible that vitamin K2 improves insulin resistence via the lipid-lowering efficacy.

FFA, free fatty acids; LC-CoA, long-chain fatty acyl-CoA.

3. The effect of different forms of vitamin K on T2DM

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As for the effect of different forms of vitamin K on T2DM, results from Beulens et al. (2010) showed that in an age-, sex-, and waist-adjusted model, vitamin K1 intake was not associated with risk of T2DM with an HR of 1.00 (95% CI 0.97–1.03) for each 50-μg increment, whereas vitamin K2 intake tended to be inversely associated (P=0.060) with risk of T2DM with an HR of 0.95 (95% CI 0.91–1.01) for each 10-μg increment. And Spline regression showed evidence of a nonlinear relation (P=0.053) between vitamin K1 intake and T2DM, whereas it showed a linear inverse association (P=0.035) between vitamin K2 intake and T2DM without evidence for a nonlinear relation[8].

As for the effect of different forms of vitamin K2 on T2DM, basically it includes fourteen compounds (MK-1 to MK-14), yet the most common ones are MK-4 and MK-7. The synthetic form of MK-4, is used in Japan as an ethical drug in the treatment of osteoporosis. MK-7, derived from the Japanese food natto (fermented soyabeans), can also be produced by variety of bacterial species, has much longer half-life in human blood compared to the other forms of vitamin K[21]. The absorption and bioavailability of MK-7 are better than MK-4. When used in the treatment of T2DM, the amount of supplemental MK-4 was in milligram (mg) dose, while MK-7 was in microgram (μg) dose, and small doses of MK-7 could produce similar effect on glucose metabolism with MK-4[9, 10].

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Disscussion

In terms of the mechanisms, there are some questions remains unsolved. Most studies in human reported that high level of carboxylated osteocalcin is associated with increased insulin sensitivity. But Iki et al. (2012) reported that serum undercarboxylated osteocalcin levels were inversely associated with glycemic status and insulin resistance[49]. Their study reoported a different result from mentioned human studies, but there is a doubt about the measurement of undercarboxylated osteocalcin in this study. They used an electrochemiluminescence immunoassay to measure undercarboxylated osteocalcin. The monoclonal antibodies used in the assay recognized OC molecules with two uncarboxylated glutamic acid residues (totally three Glu residues sides), it means the undercarboxylated osteocalcin measured in this study may contain one carboxylated residue per OC molecule. But question is that, what about OC molecule contained two carboxylated glutamic residues, how to recongnize these part of undercarboxylated osteocalcin molecules? Thus we think there are still some limitations in the measurement of the different forms of OC, carefully designed studies are needed to define the form of osteocalcin invovled in glucose gmetabolism in human.

The interpretation of this review is restricted by several factors. Firstly, this review did not tell the difference among different way of vitamin K2 supplementation, including tablet, intravenous injection and daily diet. Because vitamin K2 is heat-resistant but sensitive to light, thus if vitamin K2 is not

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properly preserved will affect the efficacy. Secondly, at present there is no recommended nutrient intake (RNI) for vitamin K2, which intakes vary widely among geographic regions, age groups and population subgroups.

The adequate intake (AI) of a nutrient is defined by specific criteria of adequacy, which is supposed to be the adequate amount based on the observation of apparently healthy people[50]. The AI values for menaquinone intake have been estimated from the UK National Dietary and Nutrition Survey, and the established amount is 54mg/d for men and 36 mg/d for women[51]. There is so far no adverse effect associated with menaquinone reported in the literature, including both animal and clinical studies. The Institute of Medicine at the US National Academy of Sciences (NHANES III) (1988–1994) has indicated as well there was no documented case of toxicity in humans or animals associated with the consumption menaquinone from diet or supplements[50].

According to current studies, vitamin K2 has a wider range of effects besides coagulation and functioning as a cofactor for GGCX. The effect on bone is to prevent and treat osteoporosis; the effect on the cardiovascular system is to prevent and treat vascular calcification; the effect on endocrine systerm is to prevent and treat T2DM. In general, vitamin K2 is a potentially therapeutic strategy in the future. This review provided an overview of the currently available studies to assess the effect of vitamin K2 supplementation on insulin sensitivity, glycaemic control and reviewed the underlying mechanisms. Overall, carboxylation of vitamin K-dependent protein osteocalcin,

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anti-inflammatory property, and lipid-lowering effect were three mechanisms underlying vitamin K2 reduced risk of T2DM. And vitamin K2 had a better effect than vitamin K1 on T2DM. The interpretation of this review will increase comprehension of the development of a therapeutic strategy to prevent and treat T2DM.

Acknowledgements

Funding: This work was supported by the National Nature Science Foundation of China [No. 30971065]; Science and technology plan of Dalian [No. 2012E12SF074]; The Education fund item of Liaoning province [No. 2009 A 194].

Declaration of interest

Conflicts of interest: none.

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Study/Study design	Participants	Exposure/Intervention	Outcomes
Beulens et al. (2010) Cohort study [8]	Participants were from the the Prospect-EPIC and MORGEN-EPIC cohorts. Between 1993 and 1997 17,357 women aged 49–70 years living in Utrecht and vicinity and 22,654 adults aged 21–64 years selected from random samples of the Dutch population in three Dutch towns were enrolled into the study. Exclusion criteria were dietary intake <600 kcal or >5,000 kcal/day, did not fill in the questionnaire, did not consent to linkage with vital status registries, reported diagnosis of prevalent diabetes. Final study population was 38,094.	A validated FFQ was used to assess levels of vitamin K1 and vitamin K2 (subtypes MK-4 to MK-10) at baseline which measured habitual consumption frequency during the past year. The relative validity of the FFQ had a Spearman's Correlation Coefficient of 0.24 for vitamin K1 and 0.51–0.72 for vitamin K2 (for subgroups MK-4 to MK-9). The validity of the FFQ was estimated against 12 monthly 24h recalls in 58 women.	Menaquinones intake tended to be inversely associated (P = 0.060) with risk of type 2 diabetes with an HR of 0.95 (95%CI 0.91–1.01) for each 10μg increment in an age-, sex-, and waist-adjusted model. In the final multivariate model, an inverse association (P = 0.038) was observed with an HR of 0.93 (0.87–1.00) for each 10μg increment of menaquinones intake. Spline regression showed a linear inverse association (P = 0.035) between menaquinones intake and type 2 diabetes without evidence for a nonlinear relation.
Choi et al. (2011) Placebo-Controlled trial[9]	Participants were volunteered, 42 healthy young male aged 29 years were enrolled into the trial. Exclusion criteria were frequently sampled intravenous glucose tolerancetest failures and extreme outliers. 18 subjects in the treatment group and 15 subjects in the control group were finally analyzed.	The treatment group received vitamin K2 (menatetrenone; 30 mg/d), while the control group received placebo t.i.d. for 4 weeks.	Vitamin K2 supplementation significantly increased S_i (4.4 v. 6.6, P = 0.01) and DI (2,266 v. 3025, P < 0.01), but these indices were not affected by placebo treatment. Treatment with vitamin K2 decreased ucOC (0.9 v. 0.4 ng/mL, P = 0.02) and increased cOC (9.6 v. 16 ng/mL, P = 0.01).
Zatollah et al. (2016) Randomised, double-blind, placebo-controlled trial[10]	At the onset of the study, patients were first matched one by one according to age, BMI, sex and the dosage and kind of medications used. Next, the matched patients were randomly assigned to the intervention and placebo groups. Inclusion criteria were overweight patients (BMI⩾25kg/m^2) with T2DM, aged 40–85 years with a CHD condition. Final study population was 66.	The treatment group received 5μg of vitamin D and 90μg of vitamin K2 to form MK-7 and 500mg Ca supplements as a tablet, while the control group received daily placebo tablets for 12 weeks.	Changes in serum insulin concentrations (−0.9 v. +2.6, P=0.01), HOMA-IR (−0.4 v. +0.7, P = 0.01), HOMA-B (−2.1 v. +8.9, P = 0.01) and the QUICKI (+0.007 v. −0.006, P = 0.01) in supplemented patients were significantly different from those in patients in the placebo group.

Table 1: The outcome of different studies on the effect of vitamin K2 on T2DM. Si: insulin sensitivity index; DI: disposition index; HOMA-IR: homoeostasis model for assessment of estimated insulin resistance; HOMA-B: β-cell function; QUICKI: quantitative insulin sensitivity check index

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Study	Object of study	Results (Osteocalcin)
Ferron M et al. (2008) [52]	WT Mice	Cell-based assays showed that picomolar amounts of osteocalcin are sufficient to regulate the expression of the insulin genes and β-cell proliferation markers, whereas nanomolar amounts affect adiponectin and Pgc1αexpression in white and brown adipocytes, respectively. In vivo the same difference exists in osteocalcin's ability to regulate glucose metabolism on the one hand and affect insulin sensitivity and fat mass on the other hand.
Pittas A et al. (2009) [16]	Adults age 65 and older	In cross-sectional analyses, serum osteocalcin concentration was inversely associated with FPG (P=0.01), fasting insulin (P=0.006), HOMA-IR (P=0.002), high-sensitivity C-reactive protein (P=0.01), IL-6 (P=0.02), BMI (P<0.001), and body fat (P<0.001).
Saleem U et al. (2010) [17]	1284 blacks and 1209 non-Hispanic whites	Osteocalcin levels were inversely correlated with body mass index, fasting glucose and insulin, HOMA-IR, triglycerides, and leptin, and positively correlated with adiponectin (P<0.001).

Table 2(A): The role of osteocalcin in T2DM on glucose metabolism in human and animal models.

Study	Object of study	Results	
		carboxylated osteocalcin (cOC)	uncarboxylated osteocalcin (ucOC)
Lee NK et al. (2007) [15]	Mice		Following a 15 min. incubation period 90% of OC present in the serum of WT mice was bound to HA whereas only 74% using serum from Esp −/ − mice. This experiment suggested that OST-PTP influences OC function by regulating its degree of γ-carboxylation and that it was ucOC that regulates glucose homeostasis.
Shea MK et al. (2009) [19]	Older men and women	HOMA-IR was lower across the higher tertiles of total OC and cOC (P=0.006 and 0.02, respectively).Those in the higher tertiles of cOC had lower fasting glucose and higher adiponectin (both P=0.03).The concentration of cOC at baseline was inversely associated with a 3-y change in HOMA-IR (P=0.002).	Lower circulating ucOC was not associated with higher HOMA-IR at baseline or at 3-y follow-up.
Hwang Y et al. (2009）[20]	Middle-aged male subjects	The upper cOC tertile was associated with lower HOMA-IR values, which are representative of insulin resistance (3.38±0.19, P<0.05).	The upper ucOC tertile was associated with higher HOMA-B% levels, which are representative of β-cell function (81.1±7.4, P<0.05).
Pollock NK et al. (2011) [22]	Overweight children	In both the normal-glucose and prediabetes groups, cOC was associated with insulin sensitivity (β=0.26, 0.47, respectively, both P<0.02).	The lower ucOC concentrations found in children with prediabetes may be associated with β-cell dysfunction.
M Iki et al. (2012) [49]	Japanese men		Levels of undercarboxylated OC, but not intact OC, were inversely associated with glycemic index and insulin resistance in a population of Japanese men.

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Table 2(B): The role of carboxylated/ uncarboxylated /undercarboxylated form of osteocalcin in T2DM on glucose metabolism in human and animal models.

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Highlights

1. Studies showed vitamin K2 intake reduced 7% T2DM risk with each 10-μg increment.
2. Vitamin K2 has a more significant effect than vitamin K1 on T2DM.
3. Vitamin K2 increased insulin sensitivity via osteocalcin metabolism.
4. Vitamin K2 improved IR via anti-inflammatory property and lipid-lowering effects.
5. Vitamin K2 suppresses inflammation via inactivating NF-κB signalling pathway.
6. Vitamin K2 supplementation decreases fat accumulation and serum triglycerides.

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