ALINEA

NUTRITION

OCTOBER 2020

www.alineanutrition.com

TABLE OF

CONTENTS

What We Know, Think We Know, or Are Starting to Know 03

The Study 04

Critical Breakdown 05

Key Characteristic 05

Interesting Finding 06

Relevance 06

Application to Practice 07

References 07

Karamzad N, Faraji E, Adeli S, Carson-Chahhoud K, Azizi S, Gargari BP. Effects of MK-7 supplementation on glycemic status, anthropometric indices and lipid profile in patients with type 2 diabetes: A randomized controlled trial. Diabetes, Metab Syndr Obes Targets Ther. 2020;13:2239–49.

What We Know, Think We Know, or Are Starting to Know

When do we ever talk about vitamin K? Named after the German word, 'koagulation', the nomenclature of vitamin K reflects its original discovery as an essential regulator of blood clot formation, i.e., coagulation.

The role of vitamin K* in blood coagulation is merely the tip of the iceberg for the biological activity of this fat-soluble vitamin, which extends to bone formation proteins, prevention against soft tissue calcification, particularly of blood vessels, and regulation of certain cellular growth and repair processes [(1)].

Vitamin K, like other fat soluble vitamins [in particular vitamin E] is an umbrella term for a number of isoforms, with vitamin K1/phylloquinone, and vitamin K2/menaquinone. Vitamin K2, however, is not a single compound, but an umbrella term for a group of vitamin K isoforms known as menaquinones. Menaquinones are synthesised by gut bacteria from phylloquinone [vitamin K1], and also produced by bacteria during fermentation of foods, resulting in a direct dietary source from consumption of certain fermented foods [(1)].

There are a range of menaquinones, from MK-2 to MK-14; the numerical spectrum reflects the number of 5-carbon units that form side-chains to the molecule [(2)]. In fact, we owe much of our understand of the metabolic effects of vitamin K2 from Japanese research groups, as the isoform MK-7 is uniquely found in high amounts in fermented soy products, in particular natto [(3)].

Specifically, MK-7 from natto consumption has been significantly associated with reduced risk of fracture and preservation of bone mineral density in a number of Japanese prospective cohorts [(3,4)]. One mechanism for this protective effect of vitamin K on bone may be osteocalcin, a bone-formation protein which is vitamin K-dependent [(5)].

However, osteocalcin also has some fascinating effects beyond bone formation. Cell culture and rodent studies have demonstrate that osteocalcin stimulates pancreatic beta-cell expression, and expression of adiponectic, a hormone derived from adipocytes [fat cells] which has insulin-sensitising effects, resulting in preserved glucose tolerance in mice [(6)]. A cross-sectional study indicated that in participants with overweight/obesity, higher osteocalcin levels were associated with preserved skeletal muscle insulin sensitivity [(7)].

One trial previuously found that MK-4 supplementation increased insulin sensitivity over 4-weeks in otherwise healthy males [(8)]. However, to date MK-7 had not been investigated for its potential anti-diabetic effects. The present study tested the effects of MK-7 on metabolic parameters in participants with type-2 diabetes.

*Geek Box: The Vitamin K Cycle

Yes, vitamin K has it' s own 'cycle' , which makes it one of those cooler nutrients. Vitamin K differs to other fat-soluble vitamins, which are generally stored in the body long-term, and the body stores very little vitamin K. As a result, in the absence of dietary intake, vitamin K can deplete to deficiency. The vitamin K cycle provides the body with a mechanism to recycle vitamin K, allowing small amounts to be used over and over. In tis cycle, vitamin K is oxidised to form vitamin K epoxide. This allows the enzyme for which vitamin K is a cofactor - γ-glutamylcarboxylase (GGCX) - to convert the amino acid glutamic acid (Gla) into y-carboxyglutamic acid [Gla], which is important for blood clotting factor proteins. Vitamin K epoxide can in turn be recycled back to hydroquinone, which is the reduced form, and is then available for further cycling. You may have heard of the anticoagulant drug, warfarin: this drug acts as a vitamin K antagonist by inhibiting the activity of one of enzymes which recycles vitamin K, thus preventing the cycle from producing proteins necessary to form blood clots.

The Study

The study was designed as a parallel arm, double-blind, placebo-controlled randomised trial in 60 men and women aged 20-55yrs with diagnosed type-2 diabetes [T2DM]. Participants were taking anti-diabetic drugs, but were not taking insulin therapy.

Participants were randomised to either the MK-7 supplement or a placebo, with identical labelling. Following randomisation, baseline blood samples for metabolic markers [blood glucose, cholesterol, triglycerides, hemoglobin A1c [HbA1c] fasting insulin), blood pressure measures, anthropometrics, and homeostatic model of insulin resistance [HOMA-IR] was calculated from fasting insulin and blood glucose measures.

Food records were completed for 3 nonconsecutive days [two weekdays and one weekend], every 3-weeks, for the duration of the 12-week intervention. Participants were given dietary advice specific to Asian patients with T2DM.

The intervention group received 200mcg/d MK-7, while the placebo was encapsulated cornstarch, and both groups were instructed to take the respective capsules with their main meal.

Results: 45 participants completed the study [23 in the intervention group, 22 in the control group]. Mean age was 43.3yrs in the intervention group and 46.9yrs in the control group, and 30% of both groups were female. There were no significant differences between groups in baseline characteristics.

There was a significant improvement in the following biomarkers of glycaemic regulation in the MK-7 group after 12-weeks supplementation, compared to the placebo group:

> Glycaemic Markers

- ***Fasting Blood Glucose:*** Decreased by significantly by 15.69mg/dL in the MK-7 group, while increasing insignificantly by 7.18mg/dL in the placebo group.

- ***Fasting Insulin:*** Decreased significantly by 5.8pmol/L in the MK-7 group, while increasing insignificantly by 1.2pmol/L in the placebo groom.
- ***HbA1c:*** Decreased significantly by 1.03% in the MK-7 group, while increasing insignificantly by 0.15% in the placebo group.
- ***HOMA-IR:*** Decreased by a score of 2.88 in the MK-7 group, while decreasing by 0.34 in the placebo group.

Adjusting for differences in baseline levels between groups and changes in vitamin K intake, neither the change in fasting insulin or HOMA-IR remained significant. However, the 24.3mg/dL lower fasting glucose in the MK-7 group, and the 1.23% lower HbA1c levels, remained statistically significant between groups [more under ***Interesting Finding,*** below].

The study found no significant differences in any of the lipid parameters measured, or in anthropometric measurements, or self-reported dietary intake, between groups. However, there was a significant increase in daily vitamin K intake of 63mcg/d in the placebo group, suggesting increased dietary intake.

Critical Breakdown

Pros: This was the first study to directly test the effects of the vitamin K2 isoform, MK-7, on metabolic parameters. Randomisation and allocation was concealed from participants and researchers during the study were achieved, and the intervention supplement and placebo were delivered in identically labelled containers. The study was conducted in a clinically representative sample of patients with T2DM.

Cons: The paper didn' t actually state what the primary or secondary outcomes were, despite giving details about sample size calculations; i.e., what was the sample size trying to detect a difference in? This is unspecific and opens the door to cherry-picking amongst findings. There was no test for baseline vitamin K2 status, but vitamin K was calculated from dietary records [although this is not explicitly stated, and would likely reflect phylloquinone intake]. The control group significantly increased dietary vitamin K level, and while there were no significant between-group differences, this may have influenced the magnitude of effect between groups.

Key Characteristic

Use of MK-7 as the supplemental intervention makes this study a 'first-in-man' , to borrow a term from pharmaceutical trials. Supplemental MK-7 has been tested before in relation to bone health outcomes, but not in relation to diabetic and metabolic parameters. However, despite the use of MK-7 as a supplement, the baseline quantification of vitamin K status and dietary assessment makes it hard to tease out effects of MK-7 itself. Previous research on MK-4 [8] is difficult to compare, given that they have divergent mechanisms of action and the half-life of MK-7 is greater than MK-4, because of its longer chain length [2]. It is is also important to note that supplemental MK-7 in the dosage range utilised in this study has been shown to increase MK-7 levels in plasma, but not increase phylloquinone levels [9]. Thus, the assessment of vitamin K in the study of dietary intake - presumable phylloquinone - does not tell us about changes in MK-7 status, and potential relationship with observed effects.

Interesting Finding

The fact that there was no change in the insulin parameters [fasting insulin and HOMA-IR], but statistically significant differences in the glucose parameters [fasting glucose and HbA1c], is interesting when we have regard to some of the animal model and limited human data which preceded this study. First, in mice ostecalcin has been shown to improve glucose tolerance [(6)]. Secondly, in a cross-sectional study comparing overweight/obese adults with impaired fasting glucose vs. normal fasting glucose, osteocalcin was associated with better glucose tolerance, but not HOMA-IR [(7)]. Thirdly, in a human supplemental intervention, MK-4 improved post-prandial glucose in response to a tolerance test, but only in participants with low vitamin K levels at baseline [(8)]. Finally, phylloquinone itself has been associated with improved insulin sensitivity [(10)]. It may be that there are effects on blood glucose regulation specific to MK-7, but the mechanisms remain to be further investigated in human research.

Relevance

The present study can be considered a pilot study, and a degree of leeway given to certain methodological limitations detailed under Cons [above], i.e., there are sufficiently encouraging findings in relation to markers of glycaemic control to warrant further investigation in a larger study with improved methodology. Thus, studies like this remain important to appropriately contextualise: it is hypothesis generating and exploratory, not explanatory or confirmatory. Viewed in this context it provides preliminary evidence of an anti-diabetic effect for vitamin K, which has previously been based largely on animal studies, with limited human data. Nonetheless, an encouraging finding was that the reduction in HbA1c reduction was sufficient to bring this marker out of diabetic range [<6.5%], which is clinical meaningful as well as statistically significant.

This study ultimately raises more questions than answers, which is fine, but important to contextualise. A previous study using MK-4 supplementation found improved insulin sensitivity after 4-weeks, which was related to increased osteocalcin [(8)]. In the present study, ostecalcin was not measured, so we do not what effect MK-7 may have had, if any, on this protein.

The fact that the effect in the present study related specifically to glucose tolerance, not insulin sensitivity, may reflect an effect of MK-7 independent of phylloquinone. However, the assessment of vitamin K status was inadequate in this study, and we can only infer that the dietary intake measured phylloquinone. Thus, we can' t even say whether the outcomes on glucose tolerance related to increased phylloquinone per se or MK-7 supplementation.

The baseline levels of vitamin K in the intervention group were at the lower end of the recommended daily intake, and it would have been useful to provide dietary instructions to avoid foods rich in vitamin K1 for the duration of the intervention, as baseline vitamin K status modifying post-prandial insulin levels in a previous study using supplemental MK-4 [(8)].

Thus, the study is preliminary and further research testing MK-7 specifically, with better methodology, will be required to elucidate the relationship between MK-7, osteocalcin [if this is the mediating mechanism of action], and glycaemic control in patients with T2DM.

Application to Practice

As a study yielding preliminary human data, there is nothing that may be applied immediately, this study is a case of the adage: "*more research needed*". Nonetheless, it is worth bookmarking this are of research to see if further studies confirm efficacy for MK-7 [or other vitamin K-2 isoforms] in the management of T2DM.

References

1. Erdman J, Macdonald I, Zeisel S. Present Knowledge in Nutrition. Oxford, UK.: Wiley-Blackwell; 2012.
2. L. Booth S. Vitamin K: food composition and dietary intakes. Food & Nutrition Research. 2012;56(1):5505.
3. Fujita Y, Iki M, Tamaki J, Kouda K, Yura A, Kadowaki E et al. Association between vitamin K intake from fermented soybeans, natto, and bone mineral density in elderly Japanese men: the Fujiwara-kyo Osteoporosis Risk in Men (FORMEN) study. Osteoporosis International. 2011;23(2):705-714.
4. Kaneki M, Hedges S, Hosoi T, Fujiwara S, Lyons A, Crean S et al. Japanese fermented soybean food as the major determinant of the large geographic difference in circulating levels of vitamin K2. Nutrition. 2001;17(4):315-321.
5. Tsukamoto Y, Ichise H, Kakuda H, Yamaguchi M. Intake of fermented soybean (natto) increases circulating vitamin K 2 (menaquinone-7) and γ-carboxylated osteocalcin concentration in normal individuals. Journal of Bone and Mineral Metabolism. 2000;18(4):216-222.
6. Lee N, Sowa H, Hinoi E, Ferron M, Ahn J, Confavreux C et al. Endocrine Regulation of Energy Metabolism by the Skeleton. Cell. 2007;130(3):456-469.
7. Gower B, Pollock N, Casazza K, Clemens T, Goree L, Granger W. Associations of Total and Undercarboxylated Osteocalcin With Peripheral and Hepatic Insulin Sensitivity and β-Cell Function in Overweight Adults. The Journal of Clinical Endocrinology & Metabolism. 2013;98(7):E1173-E1180.
8. Choi H, Yu J, Choi H, An J, Kim S, Park K et al. Vitamin K2 Supplementation Improves Insulin Sensitivity via Osteocalcin Metabolism: A Placebo-Controlled Trial. Diabetes Care. 2011;34(9):e147-e147.
9. Theuwissen E, Cranenburg E, Knapen M, Magdeleyns E, Teunissen K, Schurgers L et al. Low-dose menaquinone-7 supplementation improved extra-hepatic vitamin K status, but had no effect on thrombin generation in healthy subjects. British Journal of Nutrition. 2012;108(9):1652-1657.
10. Yoshida M, Booth S, Meigs J, Saltzman E, Jacques P. Phylloquinone intake, insulin sensitivity, and glycemic status in men and women. The American Journal of Clinical Nutrition. 2008;88(1):210-215.