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Vitamin K2 (Menaquinone-7) Supplementation and its Effect on Glucose Tolerance Test in Healthy Volunteers

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Abstract

Background: Type 2 diabetes can often be managed by healthier diet and exercise in early stages of the disease, but as it progresses oral medication is needed and later on the patients will require insulin to survive. Vitamin K is a fat soluble vitamin that is a cofactor in gamma-carboxylation and activation of coagulation proteins produced in the liver. There are also extrahepatic proteins named Gla proteins that require vitamin K to be activated. Previous studies suggest that some of these proteins have a connection to calcification of vessels, bone mineralization, metabolic syndrome and diabetes, but results are conflicting.

Aims: To study if vitamin K2 MK-7 improves glucose tolerance in healthy non-diabetic volunteers. A secondary aim was to study K-vitamin effects on coagulation, especially from safety aspects - e.g. not to induce hypercoagulation.

Research question: Can an adequate dose of 400 microgram of MK-7 for seven days affect the blood glucose tolerance test in healthy non-diabetic volunteers? Does it affect coagulation?

Methods: Blood glucose tolerance where evaluated in five healthy voluntary men by an oral glucose tolerance test before and after seven days of MK-7 supplement. Venous blood was sampled from an indwelling brachial vein catheter. Fresh whole blood was used for glucose analyses with a HemoCue Glucose 201™ at 30 min, 60 min, 90 min and 120 min after 75 gram glucose oral intake. Citrated whole blood was simultaneously analyzed on a viscoelastic global coagulation tests ROTEM®.

Results: Glucose tolerance was not improved by one week of MK-7 supplement. There were no signs of MK-7 induced hypercoagulation.

Conclusions: Our small phase I pilot study did not indicate an improved glucose tolerance or any negative coagulation effects. Next step is to study MK-7´s effect on glucose tolerance in type 2 diabetic patients.

Introduction

Vitamin K functions as a cofactor in the carboxylation of several proteins, collectively referred to as Gla proteins. In the absence of vitamin K, Gla proteins have decreased or altered functionality. In addition to hepatic clotting factors several extra-hepatic Gla proteins have been identified, such as matrix Gla protein (MGP), osteocalcin (OC) and growth arrest specific gene 6 protein (Gas6). These have been suggested to be involved in the development of cardiovascular disease, diabetes and cancer which has motivated several preclinical and clinical studies in recent years [1].

Diabetes and its related complications constitute a major global health challenge. In large-scale studies vitamin K intake has been associated with increased insulin sensitivity as well as decreased risk of type 2 diabetes, a more favorable lipid profile and decreased circulating inflammatory markers such as C-reactive protein (CRP) [2,3]. Furthermore, other studies indicate that a high intake of vitamin K2 is associated with more complete carboxylation of MGP and lower occurrence of the metabolic syndrome [4]. Study findings on vitamin K supplementation are scarce and contradictory, but it seems vitamin K2 is superior to vitamin K1 which may be related to its longer half-life and better bioavailability for extra-hepatic Gla proteins [5].

As vitamin K is required for activation of hepatic clotting factors a plausible risk of vitamin K supplementation is hypercoagulability and increased risk of thrombosis. In subjects on stable vitamin K antagonist (VKA) treatment doses a low as 10 micrograms MK-7 were shown to interfere with their treatment. Furthermore, subjects on VKA treatment are discouraged from eating food rich in vitamin K, such as Japanese specialty natto. However, in subjects not on oral anticoagulants MK-7 supplementation does not increase thrombosis risk even at doses above the recommended daily intake [6].

The aim of the present study was to investigate whether supplementation with 400 microgram MK-7 during one week affected glucose tolerance in healthy volunteers. As a secondary aim, thromboelastometry was used to evaluate safety of MK-7 with respect to the hemostatic system.

Material and Methods

Study design

This pilot study was approved by the regional ethical review board in Lund 2010-08-26, DNR 482-100. Healthy male volunteers were eligible for inclusion. Participants were supplemented with 400 microgram MK-7 for one week. Oral glucose tolerance tests and thromboelastometry were performed before supplementation and again after one week. Exclusion criteria were treatment with drugs that interact with vitamin K status such as warfarin or drugs that may affect glucose metabolism such as corticosteroids.

Participants were provided oral and written information about the study and were informed that they could drop out at any time. They were also informed that they should be fasting form midnight the day before the glucose tolerance tests and not use tobacco on the day of the test.

Blood sampling

Venous blood were drawn using a peripheral venous catheter. Before each sampling 5 ml blood were drawn and discarded. Following that, 1 ml of blood were applied to a HemoCue cuvette and analyzed immediately. For the ROTEM analysis a 2.7 ml citrated tube (Becton-Dickinson Vacutainer 0,109 M) was used to collect the blood. After each sampling the venous catheter was flushed with sodium chloride to avoid coagulation.

Oral glucose tolerance test (OGTT)

Before the OGTT participants were fasting and baseline plasma glucose were obtained (0 min). Participants were then instructed to drink 75g APL glucose dissolved in 250 ml water within five minutes. Blood samples for ROTEM analyses and analysis of plasma glucose were obtained after 30, 60, 90 and 120 minutes. The coefficient of variation for OGTT was 15-20%.

Hemocue

HemoCue Glucose 201+ was used to analyse plasma glucose at 0, 30, 60, 90 and 120 minutes. Disposable reagent-containing microcuvettes were filled with venous blood and analysed photometrically. The microcuvettes were stored in a fridge (4-8 C) until 30 minutes before analysis, according to the manufacturers' instructions.

Rotem

Thromboelastometry (ROTEM) is a viscoelastic point of care (POC) instrument for analyzing coagulation of whole blood. The EXTEM program was used and the following parameters (with their respective reference range) were analyzed: CT (clotting time) 42-74 seconds, CFT (clot formation time) 46-148 seconds, α-angle (clot strengthening) 63-81° and MCF (maximum clot firmness) 49-71 mm. ROTEM- EXTEM has a coefficient of variation that varies with the parameter: CT (<15%), CFT (<4%), α-angle (<3%) and MCF (<3%).

At each sampling time the four channels of the ROTEM machine were loaded with a cuvette. The reagents STARTEM and EXTEM were mixed with 0.36 ml venous blood and added to the cuvettes.

MK-7

All study participants were given 14 capsules MenaQ7 from NattoPharma (Oslo, Norway). Each capsule contained 200 microgram MenaQ7® and 10 microgram vitamin D3. Participants were instructed to take two capsules each morning during one week.

Statistics

Statistics were processed using Microsoft Excel and GraphPad Prism. The area under the curve was computed for the glucose values before and after vitamin K supplementation using a trapezoid model.

Wilcoxon's matched-pairs signed rank test was used to compare differences before and after supplementation. A p-value of <0.05 was considered statistically significant.

Results

Five participants were included in the study. One subject did not complete the study due to an allergic reaction to the venous catheter used for blood sampling. Results are therefore calculated based on the values of the remaining four participants.

OGTT before and after MK-7 supplementation

The individual glucose values of each participant were used to compute the mean value of plasma glucose at 30, 60, 90 and 120 minutes after the OGTT at baseline and after one week of vitamin K supplementation. A trapezoid model in Excel was used to compute the area under the curve (AUC) and mean values were used to calculate change over time.

There was no significant change in the AUC before or after MK-7-supplemenation (p = 0.0625). Figure 1 shows the OGTT before and after vitamin K supplementation. Individual plasma glucose values are detailed in Tables 1- 4.

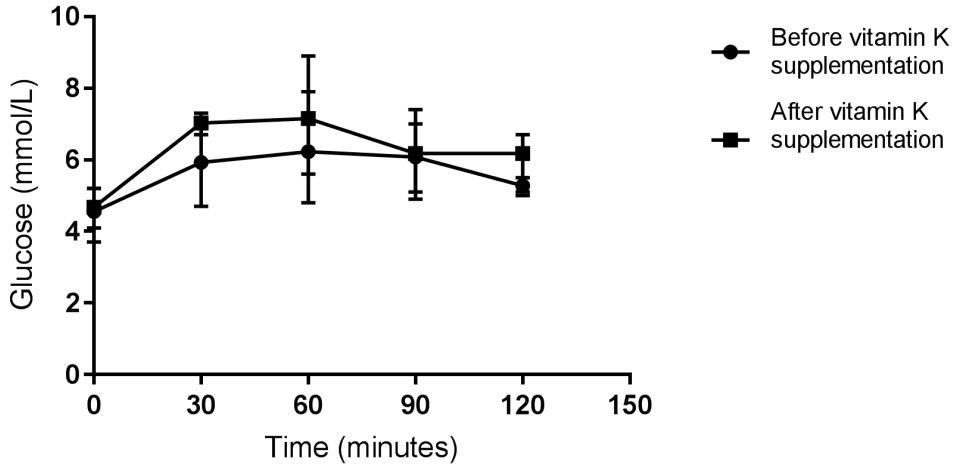

Figure 1: Distribution and mean value of plasma glucose at 0, 30, 60, 90 and 120 minutes before and after vitamin K supplementation P-value 0.0625.

Study participant	P-glucose 0min	P-glucose 30min	P-glucose 60min	P-glucose 90min	P-glucose 120min
1	5.2	6.6	4.8	5.1	5
2	4.8	6.9	5.5	6	5.5
3	5.1	7.7	7.3	5.4	6.4
4	3.7	4.7	6.7	6.2	5.2
5	4.5	5.5	7.9	7	5.4

Table 1: Venous plasma glucose (p-glucose) at 0, 30, 60, 90 and 120 minutes after OGTT before MK-7 supplementation

Study participant	P-glucose 0min	P-glucose 30min	P-glucose 60min	P-glucose 90min	P-glucose 120min
1	5.2	7.3	7.1	6.1	6.7
2	4.5	6.7	5.6	4.9	5.1
3					
4	4.1	6.9	7	6.3	6.6
5	4.9	7.2	8.9	7.4	6.3

Table 2: Venous plasma glucose (p-glucose) at 0, 30, 60, 90 and 120 minutes after OGTT after MK-7 supplementation

Time	P-glucose (Before MK-7)	Trapezoid
0	4.55	
30	5.925	5.2375
60	6.225	6.075
90	6.075	6.15
120	5.275	5.675
	Area under the curve	23.1375

Table 3: Mean value of plasma glucose (P-glucose) at 0, 30, 60, 90 and 120 minutes after OGTT before MK-7 supplementation

Time	P-glucose (After MK-7)	Trapezoid
0	4.675	
30	7.025	5.85
60	7.15	7.0875
90	6.175	6.6625
120	6.175	6.175
	Area under the curve	25.775

Table 4: Mean value of plasma glucose (P-glucose) at 0, 30, 60, 90 and 120 minutes after MK-7 supplementation

ROTEM before and after MK-7 supplementation

The individual values of CT, CFT α-angle and MCF were used to compute median values. The median was used since two CFT values were significantly deviating from the others. There was no significant change in ROTEM parameters CT, CFT, α-angle and MCF when comparing before and after one week of MK-7 supplementation. The median values for CT, CFT, α-angle and MCF are shown in Figure 2-5.

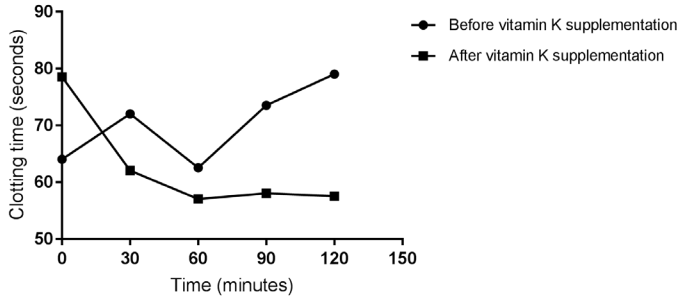

Figure 2: Median value of clotting time at 0, 30, 60, 90 and 120 minutes before and after MK-7 supplementation. P-value 0.3125

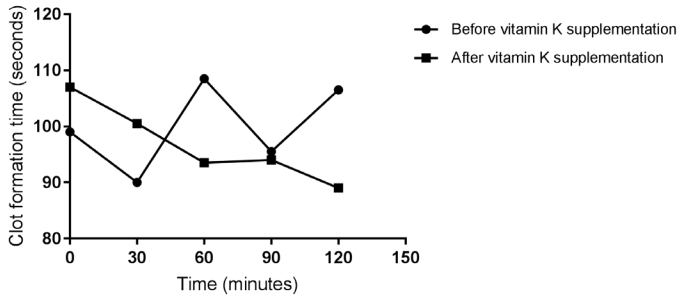

Figure 3: Median value of clot formation time at 0, 30, 60, 90 and 120 minutes before and after MK-7 supplementation. P-value 0.6250

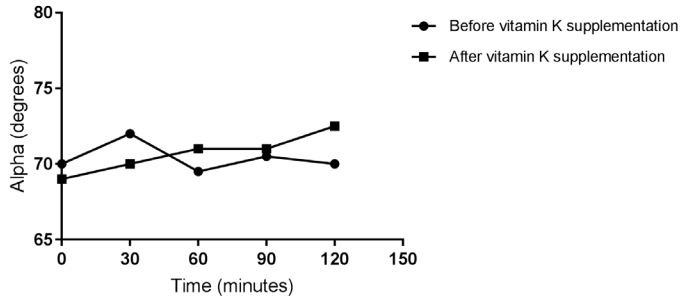

Figure 4: Median value of clot strengthening at 0, 30, 60, 90 and 120 minutes before and after MK-7 supplementation. P-value 0.8125

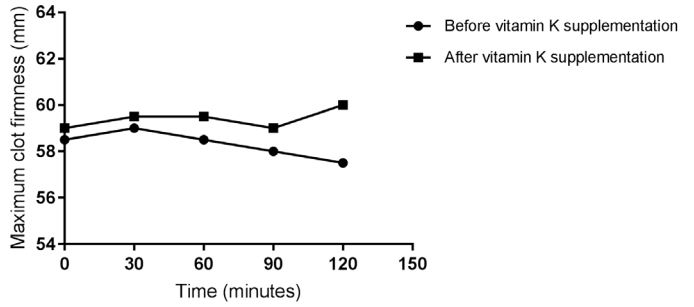

Figure 5: Median value of maximum clot firmness at 0, 30, 60, 90 and 120 minutes before and after MK-7 supplementation. P-value 0.0625

Discussion

In the present study the effect of one week supplementation with 400 microgram MK-7 on glucose tolerance and hemostatic system in healthy subjects was evaluated using OGTT and thromboelastometry. No significant changes were demonstrated, i.e. glucose tolerance was not improved and there were no adverse effects on coagulation. This is concordant with a previous similar study where 12 healthy young males were given 90 mg vitamin K2 MK-4 daily during one week and glucose loading response were evaluated before and after with no change [6]. In this study authors did demonstrate a significant decrease in immunoreactive insulin (IRI) in the participants with a high baseline serum descarboxy-prothrombin (DCP, PIVKA-II) and postulated that during vitamin K deficiency a relative glucose intolerance may be present. In the present study neither vitamin K status nor IRI were evaluated. The results from this study is also in line with previous findings where healthy subjects were given up to 360 µg MK-7 for 12 weeks with no effect on thrombin generation [7].

The existing evidence for the role of vitamin K in development of diabetes is conflicting. Some studies suggest that increased levels of vitamin K dependent protein growth arrest specific gene 6 protein (gas6) are associated with insulin resistance [8], and that genetic variations of gas6 increases the risk of the metabolic syndrome [9]. A recent systematic review concluded

that glycemic control was not significantly affected by vitamin K supplementation, but that further well-designed studies on diabetic and pre-diabetic patients are needed [10].

Limitations on the present study include a very small study population, short observation time and limited access to equipment to be able to perform more advanced tests such as plasma insulin. Furthermore, when using the OGTT plasma glucose levels change quickly, and the variation between repeated samples is relatively high [11].

One study participant could not sit and rest between the blood samples at the first sampling time. Due to this it is possible that more glucose was consumed which is reflected by the lower values (Table 1). The fasting period for study subject 4 and 5 was a few hours longer at baseline compared to the second OGTT, which might have contributed to better glucose tolerance at baseline. It is also possible that participants did not adhere to the instructions given prior to entering the study. In addition to MK-7, the capsules also contained vitamin D3. Even though the dose was relatively low, interactions cannot be excluded.

Conclusion

In this study, one week of supplementation with MK-7 to healthy young males did not affect glucose tolerance or thromboelastography. Larger studies are needed on patients with impaired glucose metabolism who are supplemented for a longer period of time.

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