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Original Contribution

EFFECTS OF VITAMIN K ON LIVER STEATOSIS AND PANCREATIC LIPOMATOSIS IN EXPERIMENTAL MODEL OF METABOLIC SYNDROME

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ABSTRACT

PURPOSE: Recently new roles for vitamin K, different from that in coagulation, have been proposed, including prevention of cardiometabolic diseases. It was the aim of the present work to evaluate the impact of vitamin K treatment on the changes in liver and pancreas of rats with experimentally induced metabolic syndrome.

METHODS: Four groups of rats were used, as follows: one control group fed regular rat chow; one group fed high fat, high fructose (HFHF) diet for 12 weeks to induce a metabolic syndrome (MS) and two groups with MS treated with vitamin K1 and K2 respectively. At the end of the experiment, liver tissue and pancreatic were dissected out for morphological examination.

RESULTS: All groups of rats fed HFHF diet expressed liver histological changes consistent with steatosis. These alterations were more pronounced in the groups treated with vitamin K2 and K1. The pancreatic tissue of the HFHF fed animals showed similar degree of lipomatosis irrespective of treatment.

CONCLUSIONS: In rats with diet-induced MS, treatment with vitamin K1 and K2 did not produce the expected morphological evidence of improvement, even tended to aggravate the liver changes. These results disagreed with other effects of vitamin K that were established.

Key words: Vitamin K, liver steatosis, pancreatic lipomatosis, rats, high-fathigh-fructosediet

INTRODUCTION

Metabolicsyndrome (MS) is a co-occurrence of at least three out of the following conditions: arterial hypertension, impaired glucose tolerance, high triglyceride and low HDL levels, abdominal obesity, insulin resistance. It increases the risk of developing diabetes type II and cardiovascular disease. Its worldwide prevalence rises constantly and new therapies and preventive strategies are being constantly sought.

Besides its important function in blood clotting, vitamin K has been recently associated with different new roles. It has been shown to be beneficial in osteoporosis treatment (1). It is recognized as a potent inhibitor of vascular calcification (2). Epidemiological and experimental evidence links vitamin K with potential for anticancer effects (3). In brain this vitamin is possibly exerting neuro-protective activity (4). Ultimately, vitamin K has been implicated in prevention of metabolic disorders (5).

There are no studies, examining the effects of vitamin K on metabolic syndrome in rats. It was the aim of this work to look at the morphological changes in liver and pancreas that characterize diet-induced MS in rats and to outline the effects of vitamin K on these changes, if any.

MATERIALS AND METHODS

Experimental drugs and substances

Vitamin K1 was purchased from Sigma Aldrich in the form of oily concentrate, which was subsequently diluted in sunflower oil down to concentration of 1.3%. Vitamin K2

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was purchased from Seebio Biotech, Inc., in the form of 1.3% oily solution of MK-7 in sunflower oil. Fructose, lard and sunflower oil were commercially available products.

Experimental animals

Male Wistar rats were used. They were divided in 4 groups of 10 animals each. MS was induced by feeding 30 rats high-fat-high-fructose diet (HFHF) and adding 10% fructose to the drinking water. The HFHF diet contained 17% lard and 17% fructose added to the regular rat chow. Ten rats of the MS group received daily (5 days in a week) vitamin K2 by intragastric gavage at a dose of 30 mg/kg; other ten rats received vitamin K1 at the same dose and manner. Since the vitamins K were dissolved in sunflower oil, the control MS group received intragastrally the vehicle, sunflower oil. The last group was a normal control group, where rats were fed regular rat chow, drank plain water and received water intragastrally. The treatment of the experimental groups can be summarized in **Table 1.**

Table 1.*Treatment of experimental groups*

Group / treatment	HFHF diet + 10% fructose in water	Vitamin K1	Vitamin K2 (MK7)	Sunflower oil
I (K2+MS)	+	-	+	-
II (K1+MS)	+	+	-	-
III (MS control)	+	+	+	+
IV (Normal control)	-	-	-	-

*****HFHF - High-fat-high-fructose diet.

The entire experiment lasted for 12 weeks. At the end of the experiment the rats were sacrificed by cervical dislocation under light ether anesthesia and the liver, retroperitoneal fat and pancreas from each animal were dissected out, dry weighed and preserved in 10% neutral formalin for histological evaluation.

The study was approved by the Bulgarian Food Safety Agency and was conducted in agreement with the National Policies and the Council Directive (86/609/EEC).

Tissue preparation and processing of histological examination

After 24 hour fixation, samples from liver and pancreatic tissues were cut into appropriate portions and placed inembedding cassettes. The process continued with dehydration in progressively more concentrated ethanol baths, followedby a clearing agent (xylene) and finally molten paraffin wax infiltrated the samples. Next steps were to embed tissues into paraffin blocks, cut 5 μm slices and mounted them on a glass microscope slide. In the end, sections were stained with hematoxylin and eosin stain.

RESULTS

The degree of liver steatosis and pancreatic lipomatosis in the rats of every experimental group was used as a marker of metabolic syndrome. **Table 2** depicts the established facts.

The liver **(Figure 1D)** and pancreatic tissues **(Figure 2D)** from the normal control rats showed scarcely perceptible alterations.

In the MS control group the liver tissue displayed microvesicular steatosis in each sample from the group seen as small vacuoles in the cytoplasm around the nucleus **(Figure 1C).**

Vitamin K1 treated rats expressed a more severe degree of liver steatosis **(Figure 1B)**. In addition to the microvesicular fatty degeneration, multivesicular steatosis was also found. These changes were located both in the periportal and centrilobular zones.

In the vitamin K2 treated rats the alterations described above were still more pronounced **(Figure 1A).**

The pancreata of the rats from the vitamin K treated groups were affected by lipomatosis evident along the blood vessels and channels, as well as between the acini. The extent of pancreatic lipomatosis was almost indistinguishable among the groups **(Figures 2A, 2B),** irrespective of the different treatments that these rats received. The MS control group presented less manifested pancreatic lipomatosis than in the first two groups **(Figure 2C).**

Table 2.*Comparison of liver steatosis and pancreatic lipomatosis*

Group	Liver steatosis	Pancreatic lipomatosis
I (MK7+HFHF*)	+ + + +	+ + +
II (K1+HFHF*)	+ + +	+ + +
III (HFHF*)	+ +	+ +
IV (Control)	+	+

*****HFHF - High-fat-high-fructose diet.

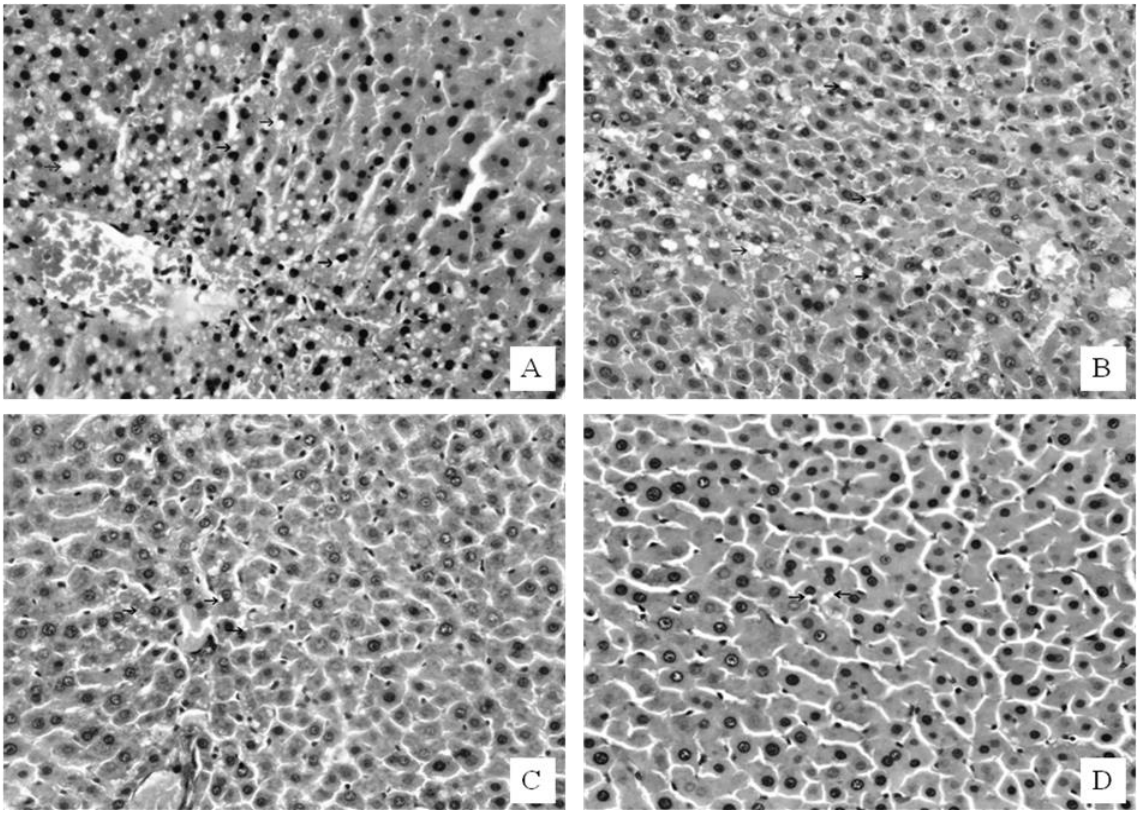

Figure 1 Liver tissue. **A,** High-power view of liver steatosis from the first group of rats.There is accumulation of fat in most of hepatocytes which is seen as small vacuoles in the cytoplasm around the nucleus (*arrows*) (H&E, x200).**B,** Second group – a smaller number of cells has fatty deposits (H&E, x200). **C,** Fatty degeneration is less prominent than alterations observed in the second group (H&E, x200). **D,** only a few hepatocytes are affected (*arrows*) (H&E, x400).

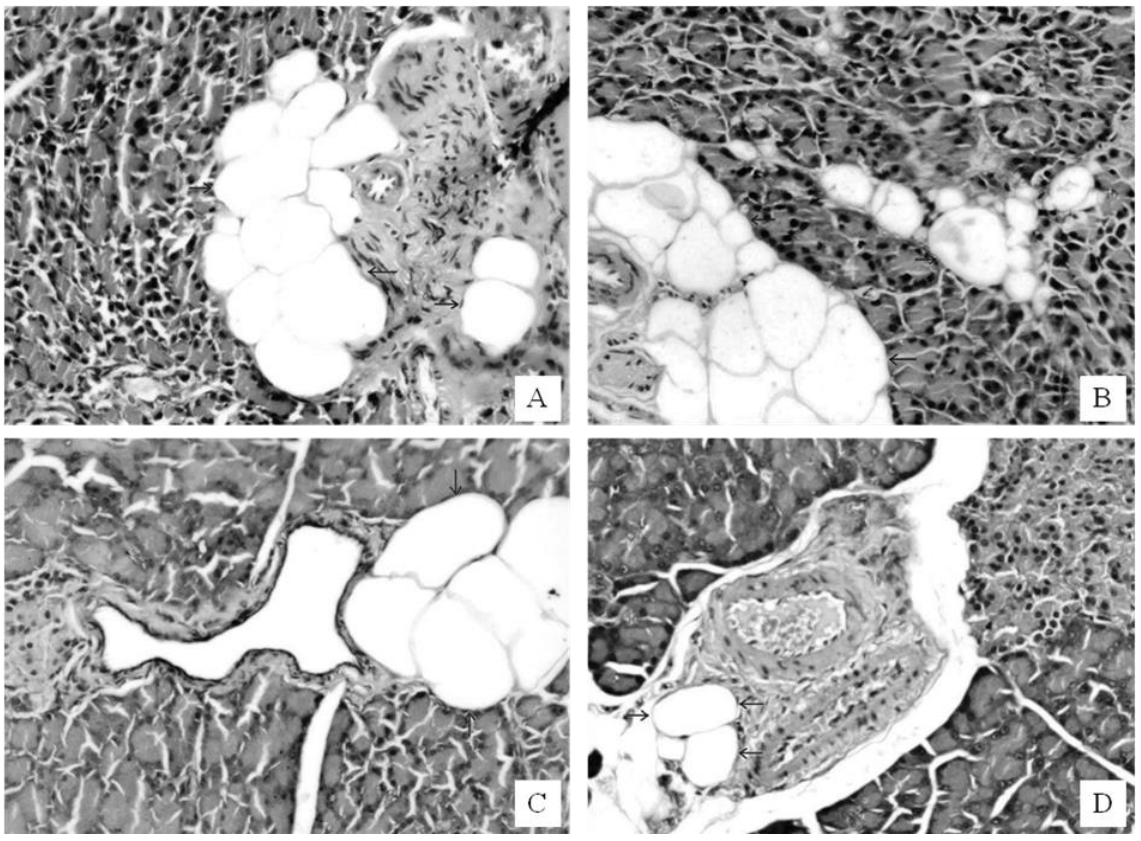

Figure 2. Pancreatic tissue. Degree of pancreatic lipomatosis in the first two groups of rats is almost indistinguishable (**A, B)** (H&E, x200). **C;** Pancreatic lipomatosis is less manifested than in the first two groups (H&E, x200). **D;** Only a few perivascular adipocytes are present (*arrows*) (H&E, x200).

DISCUSSION

As a whole, from the morphological standpoint, metabolic syndrome was mostly evident in the group of rats treated with vitamin K2, followed by the group treated with vitamin K1 and the manifestations in the control MS group were relatively modest.

These results are generally in disagreement with our expectation based on the other findings from the same experiment. The fat index (retroperitoneal fat/body weight) of rats treated with vitamin K2 was reduced with borderline significance relative to the increased fat index in the MS control group, while vitamin K1 had no such effect. As for the triglycerides, in the vitamin K2 and K1 treated groups they remained higher than the normal controls but lower than the MS control, with no significant differences from both controls. In any case the parameters characterizing the lipid metabolism in the groups receiving K vitamins were no worse than those of the MS control group.

There are no similar experimental settings reported in the literature to compare our morphological results with. The experimental studies are usually centered on bone effects of vitamin K and the biological or biochemical measures are only reported as secondary outcomes.

Sogabe et al (6) supplemented rat food with vitamin K1 and K2 for 3 months at quantities comparable to our doses, which led to decrease in total fat accumulation and serum triglycerides, along with positive effects on mineral density and strength parameters of bone. It is to note, however, that these rats were not subjected to hypercaloric diet manipulation.

Kim et. al. (7) was interested in bone indices of mice made obese by high-fat diet. In addition to the main finding that vitamin K1 and K2 supplementation reversed the high fat diet induced bone deterioration, they also report, similarly to our results, reduction of the perirenal and total body fat in the vitamin K fed animals with more pronounced effects associated with vitamin K2.

Vitamin K2 has been shown also to prevent hyperglycemia in rats treated with streptozotocin (8, 9).

Epidemiological evidence suggested that dietary phylloquinone (vitamin K1) and menaquinones (the collective name of different forms of vitamin K2) are associated with reduced risk of type 2 diabetes (10). In a large prospective study a cohort of 38094 Dutch men and women (20-70 years old) was followed up for 10 years. The results showed a tendency for vitamin K1 and a significant inverse association for the intake of menaquinones and the risk of type 2 diabetes. In another clinical study increased adiposity in older adults was associated with poorer vitamin K status (11).

There are different possible mechanisms whereby vitamin K could potentially influence energy metabolism. First of all, vitamin K is involved in gamma-glutamic carboxylation of the so called VK dependent proteins, such as coagulation factors, which are considered active after being carboxylated at their glutamic acid (GLA) residues. One of these proteins is osteocalcin, known also as bone-derived GLA protein. Although classically associated with bone formation, recently this protein was implicated as an important player in a regulatory circuitry between bone, pancreas, fat tissue and CNS. A group of researchers from Columbia University led by G. Karsenty (12) showed in a series of elegant experiments, by using genetically modified mice, that osteocalcin in its undercarboxylated form was responsible for a number of potentially beneficial effects such as insulin and adiponectin secretion, inhibition of leptin, improvement of insulin resistance. This would imply that, vitamin K, by reducing the undercarboxylated osteocalcin, would unfavorably affect glucose tolerance. Subsequent clinical experiments tried to look at this contradiction in humans, but the results so far are inconsistent and equivocal. To mention few of these:

Kumar et al., for example, found that phylloquinone administration in post meno-pausal women was not associated with changes in insulin secretion and action despite reductions in concentrations of under carboxylated osteocalcin (13). In another clinical study (14) both under carboxylated and carboxylated forms of osteocalcin were associated with different aspects of improved glucose tolerance – enhanced beta-cell function and improved insulin sensitivity, respectively. A recent clinical investigation (15) demonstrated that, phylloquinone supplementation in premenopausal pre-diabetic women significantly increased serum adiponectin concentration, did not alter total osteocalcin and leptin and improved the glycemic status.

Although diverse in respect to their results and mechanism of action, the published studies involving vitamin K supplementation in humans rarely focus on undesired or potentially

deleterious effects related to cardiometabolic disorders. One such study, however, reports unfavorable lipid effects (increased triglycerides and borderline decrease of HDL) after six week vitamin K supplementation (16). These results could be commented indirectly as potentially supporting our morphological findings.

Another possible, carboxylation-independent, connection between vitamin K and energy homeostasis, providing a clue for its potentially favorable effects, are inflammation and oxidative stress – two well characterized features of MS. High vitamin K status was associated with low level of inflammatory markers in the Framingham Offspring Study, while undercarboxylated osteocalcin was not (17). There are also data showing that vitamin K is a potent antioxidant (18).

In conclusion, the present morphological findings do not support the anticipated preventive role of vitamin K1 and K2 in diet-induced metabolic syndrome. The discrepancies with most of the literature data are difficult to explain. Further investigations are necessary to confirm and to elucidate the nature of the observed changes.

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