Chapter 10

Menaquinone-4 Enhances Steroidogenesis in Testis Derived Tumor Cells Via the Elevation of cAMP Level

Hsin-Jung Ho, Hitoshi Shirakawa and Michio Komai

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/63982

Abstract

Naturally existing vitamin K consists of vitamins K1 and K2. Menaquinone-4 (MK-4), an analog of vitamin K2 and a product of vitamin K1 metabolism, can be detected in several organs, including the testis; however, the function of MK-4 in these tissues has not been well characterized. Recent studies have suggested that vitamin K is involved in enhancing protein kinase A (PKA) activity in several cell types, thus regulating numerous PKA-dependent biological processes. To highlight the effect of vitamin K, we focused on its role in the steroidogenic pathway. Experiments on vitamin K–deficient rats revealed a reduced expression of genes involved in the biosynthesis of cholesterol and steroid hormones in the testis. Moreover, compared with control animals, rats fed on MK-4 diet presented significantly higher testosterone levels in the plasma and testis. These results suggest that vitamin K is involved in the steroidogenic pathway in the testis. Testosterone levels were found to increase in a dose-dependent manner also in cell-based experiments upon addition of MK-4, but such an effect was not observed in vitamin K1 levels. Furthermore, the effect of MK-4 on testosterone production was abolished by the specific PKA inhibitor H89, thus confirming the regulatory role of MK-4 on PKA activation. Here, we describe how MK-4 modulates PKA activation by enhancing intracellular 3′,5′-cyclic adenosine monophosphate (cAMP) levels in testis-derived I-10 cells. The presented evidence supports the role of MK-4 in cAMP/PKA signaling and steroidogenesis.

Keywords: menaquinone-4, steroidogenesis, cAMP, protein kinase A, Leydig cells

1. Introduction

Naturally existing vitamin K comprises vitamin K1 (phylloquinone) and vitamin K2 (menaquinone). Menaquinone-4 (MK-4), an analog of vitamin K2, contains a 2-methyl-1,4-naphtho-

quinone ring and a geranylgeranyl group (four isoprene units) as a side chain (**Figure 1**). MK-4 is not commonly synthesized by bacteria and is instead alkylated from menadione, which is supplemented in animal feeds to increase vitamin K levels. In most organs, MK-4 is converted from dietary vitamin K1 and other menaquinones [1, 2] via a process catalyzed by UbiA prenyltransferase domain containing protein 1 [3]. Furthermore, MK-4 is prescribed as a therapeutic agent for osteoporosis and to prevent fractures in Japan [4].

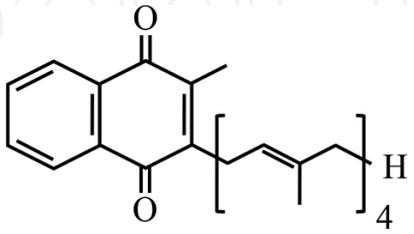

Figure 1. Chemical structure of MK-4. MK-4 has a 2-methyl-1,4-naphthoquinone ring and a geranylgeranyl group (four isoprene units) as a side chain.

Vitamin K is a well-known nutrient required for blood coagulation and bone metabolism. In recent years, novel functions of vitamin K against inflammation [5, 6], tumors [7–9], and ligand of the nuclear receptor PXR (also known as SXR) [10, 11] have been reported. These findings suggest the beneficial role of vitamin K, including MK-4, in several biological processes. In rodents, MK-4 is distributed throughout the body and is observed in high quantity in the liver, bone, brain, pancreas, and reproductive organs, even when animals are fed a low MK-4 diet (**Figure 2**) [12–15]. However, the role of MK-4 in these organs has not been well characterized. This chapter focuses on the functional effects of MK-4 on steroidogenesis in testicular Leydig cells.

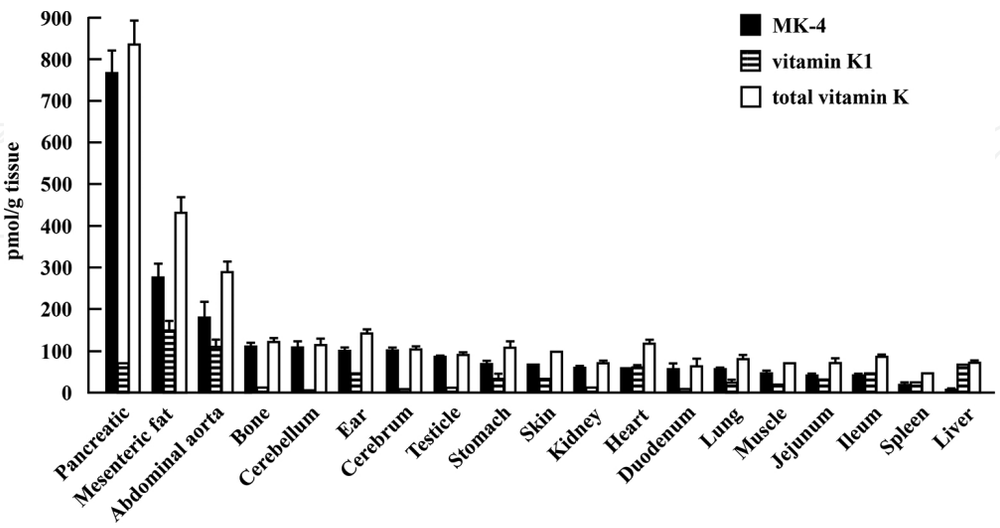

Figure 2. Vitamin K contents in rat tissues. Male Wistar rats were fed an AIN-93G diet for three weeks. Tissue levels of vitamin K and MK-4 were determined by fluorescent HPLC (reproduced with permission from Shirakawa et al. [14]).

http://dx.doi.org/10.5772/63982

2. Steroidogenesis in testicular Leydig cells

The major function of testicular Leydig cells is to produce testosterone in response to the pituitary luteinizing hormone (LH) as shown in **Figure 3**. The LH receptor (LHR) affects the 3′,5′-cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) signaling pathway by associating with G proteins containing the cytoplasmic Gαs subunit. Production and secretion of testosterone in Leydig cells are tightly regulated by intracellular cAMP, a common secondary messenger. The formation and degradation of intracellular cAMP are under the control of adenylate cyclase (AC) and cyclic nucleotide phosphodiesterase (PDE), respectively. A rise in intracellular cAMP levels activates PKA, which stimulates downstream steroidogenic proteins. Steroidogenic acute regulatory (StAR) protein transports cholesterol to the inner mitochondrial membrane of these cells to initiate steroidogenesis. Cytochrome P450scc (also known as CYP11A), a cholesterol side-chain cleavage enzyme, catalyzes a cascade of reactions that convert cholesterol to the steroid hormone precursor pregnenolone, which is then transformed into testosterone [16]. Both StAR and CYP11A constitute rate-limiting steps in the overall steroidogenesis of testosterone from cholesterol.

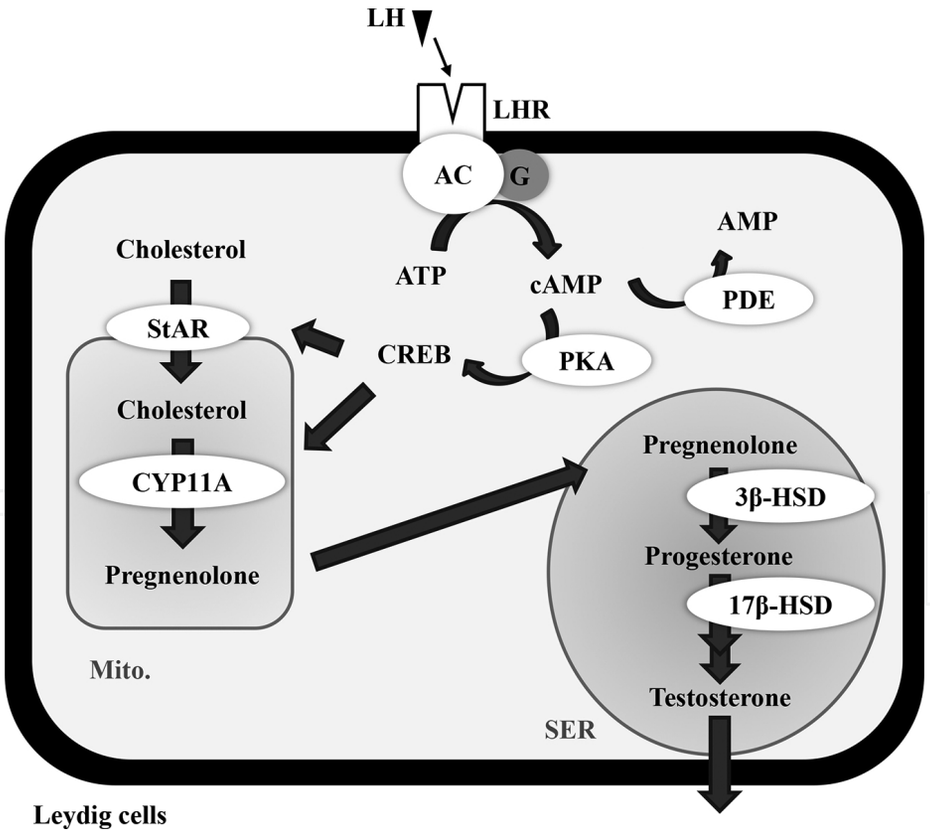

Figure 3. Steroidogenic pathway in testicular Leydig cells. AC, adenylate cyclase; CREB, cAMP response element-binding protein; CYP11A, cholesterol side-chain cleavage enzyme; G, G protein; HSD, hydroxysteroid dehydrogenase; LH, luteinizing hormone; LHR, LH receptor; Mito, mitochondria; PDE, cyclic nucleotide phosphodiesterase; PKA, protein kinase A; SER, smooth endoplasmic reticulum; StAR, steroidogenic acute regulatory protein.

Testosterone production in men is crucial for fetal development, sperm production, and the development of male secondary sex characteristics. Furthermore, increasing evidence shows that lowering testosterone production causes infertility and sexual dysfunction in men, including cases of late-onset hypogonadism (LOH) [17, 18]. The current progressive aging of the population and the stress of modern life may also contribute to LOH, resulting in sexual dysfunction, muscle weakness, and even depression. Moreover, low testosterone levels can also predict the development of several diseases, such as type 2 diabetes and cardiovascular disease [19–21]. This issue could be addressed by identifying nutrients that may boost testosterone levels.

3. Vitamin K modulates the activation of PKA in various cell types

Vitamin K has been shown to affect several biological processes by modulating the activity of PKA in different cell lines. Thus, vitamin K has been reported to enhance nerve growth factor-mediated neurite outgrowth via PKA activation in PC12D pheochromocytoma cells [22]. Vitamin K2 has also been reported to inhibit growth and invasion of hepatocellular carcinoma cells through the activation of PKA, which modulates the activities of several transcriptional factors and inhibits the small GTPase Rho [23]. Furthermore, vitamin K2 has been shown to modulate gene expression in osteoblasts upon activation of PKA [24]. Taken together, these findings indicate that vitamin K plays an important role in the activation of PKA in various cell types.

4. MK-4 enhances steroidogenesis by activating PKA

We confirmed the link between vitamin K and steroid production using DNA microarray analysis. We observed that the expression of genes involved in the biosynthesis of cholesterol and steroid hormones was decreased in vitamin K–deficient rats. The mRNA level of Cyp11a positively correlated with MK-4 concentration in the testis. Moreover, testosterone levels in the plasma and testis of vitamin K–deficient rats were significantly reduced, in spite of normal levels of plasma LH [25]. Another study further described the effects of dietary vitamin K on testosterone production. Rats fed on MK-4-supplemented diet for five weeks presented significantly higher plasma and testis testosterone levels compared to those of control rats, irrespective of changes in plasma LH levels [26]. Moreover, Cyp11a protein levels in the testis were higher in the MK-4-supplemented group than in the control. These results suggest that vitamin K is involved in steroid production in the testis. To link these results with the anti-inflammatory properties of vitamin K observed in the lipopolysaccharide (LPS)-induced models [5, 6], we examined the effects of dietary vitamin K on steroidogenesis in LPS-induced rats [27]. We found that dietary vitamin K intake affected testicular vitamin K concentration and offset the LPS-induced lowering of testosterone synthesis in the testis. In summary, testicular vitamin K plays an important role in steroidogenesis in Leydig cells.

http://dx.doi.org/10.5772/63982

We also found a relationship between vitamin K and steroidogenesis in testis-derived tumor cells. After incubation of two testis-derived cell lines, mouse I-10, and rat R2C, in the presence of MK-4, we detected a dose-dependent rise in secreted testosterone in culture medium [26]. In I-10 cells, MK-4, but not vitamin K1, led to increased levels of testosterone. We also found that menaquinone-3 and menaquinone-7 stimulated testosterone production in I-10 cells (unpublished data). These results suggest that menaquinones, which have an unsaturated isoprenyl side chain, enhance testosterone production. In I-10 cells, the production of progesterone, a testosterone precursor, increased in a dose-dependent manner following treatment with MK-4. This indicates that stimulation of steroidogenesis by MK-4 occurs upstream of progesterone synthesis (**Figure 2**). To assess the effects of MK-4 on the activation of PKA in I-10 cells, a reporter gene assay was employed (**Figure 4**). The cAMP response element-driven luciferase reporter plasmid was transfected into I-10 cells. Accordingly, MK-4 enhanced reporter activity relative to the control. Moreover, Western blot analysis revealed that MK-4 increased the expressions of Cyp11a, as well as phosphorylation levels of PKA and the cAMP response element-binding protein (CREB) in I-10 cells. In contrast, the increase in testosterone level induced by MK-4 was completely abolished by treatment with the PKA inhibitor H89 [26]. These results indicate that, unlike vitamin K1, MK-4 significantly stimulates testosterone production and may play an important role in steroidogenesis.

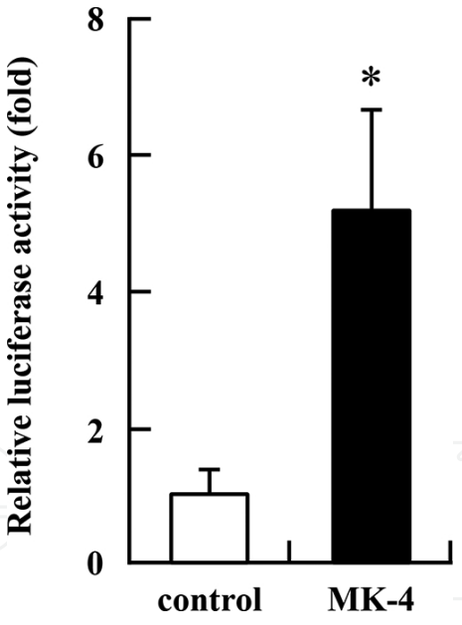

Figure 4. PKA activation following treatment with MK-4 in I-10 cells. I-10 cells were transfected with a plasmid bearing a cAMP response element fused to a luciferase reporter gene and then treated with MK-4.

Our findings were in accordance with previous studies by Ichikawa et al. [24], who showed that MK-4, but not vitamin K1 or other vitamin K2 isoform, specifically induced mRNA levels of GDF15 and STC2, whose protein levels are regulated by PKA in human and mouse osteoblasts. However, Tsang and Kamei have reported that both vitamin K1 and MK-4 promote nerve growth factor-dependent outgrowth of neuronal cells, which were blocked in the

presence of a PKA inhibitor [22]. The inconsistencies between these studies may be explained by differences in the uptake, metabolism, and solubility of each vitamin K analog used. In one example, MK-4 was taken up faster than vitamin K1 by MG-63 osteosarcoma cells and HepG2 hepatoma cells by using stable isotope-labeled vitamin K1 and MK-4 [28].

In contrast to a report that MK-4 activated PKA without increasing intracellular levels of cAMP in hepatocellular carcinoma cells [23], we showed that intracellular cAMP levels increased in a dose-dependent manner by treatment with MK-4 for 1.5 h in I-10 cells (**Figure 5**). These results indicated that MK-4 enhances testosterone and progesterone production via activation of PKA as well as modulation of cAMP levels in testis cells.

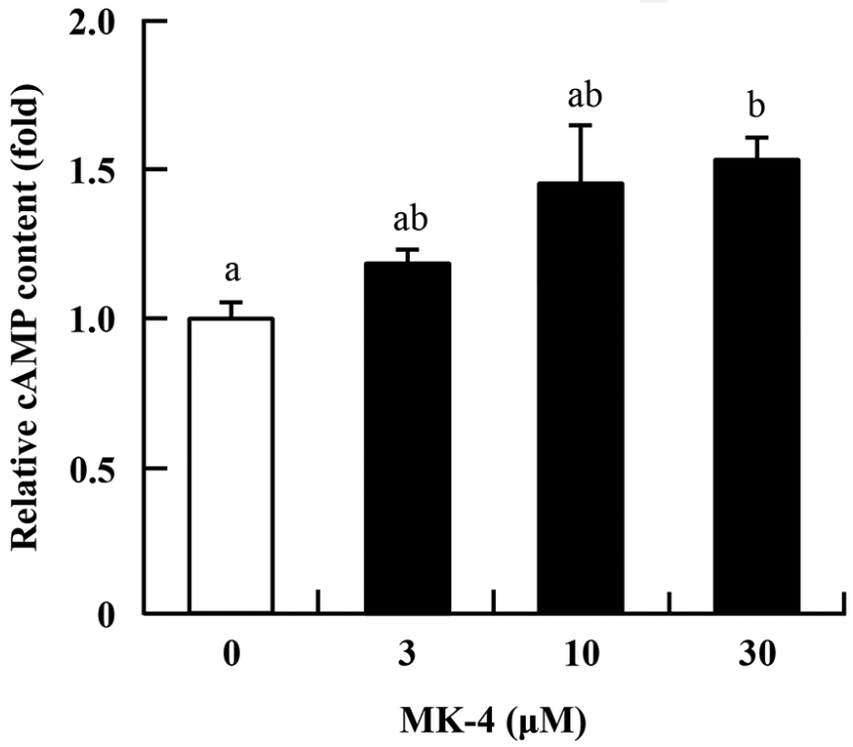

Figure 5. Intracellular cAMP levels in I-10 cells upon MK-4 treatment.

5. The MK-4 structurally related compound, geranylgeraniol

The C20 isoprenoid compound, geranylgeraniol (GGOH, **Figure 6**), is a functional side-chain component (the geranylgeranyl group) of MK-4 and has been shown to have a similar biological function as MK-4. In addition, GGOH may also have anti-tumorigenic effects against prostate cancer [29], colon cancer [30], leukemia [31], as well as anti-inflammatory activity in rats [32]. Our latest study revealed a time- and dose-dependent increase in testosterone and progesterone production in GGOH-treated I-10 cells. As expected, addition of GGOH stimulated also the PKA signaling pathway and augmented intracellular cAMP levels in I-10 cells [33].

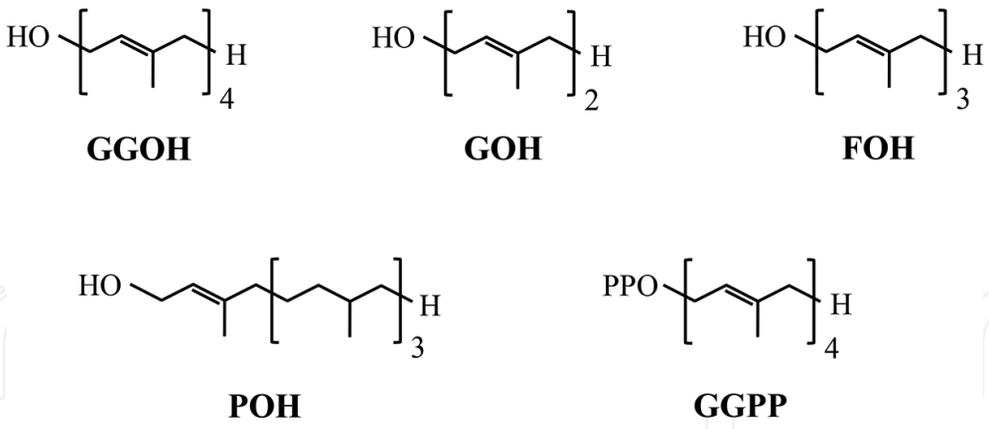

Figure 6. Chemical structures of isoprenoid groups; geranylgeraniol (GGOH), geraniol (GOH), farnesol (FOH), phytol (POH), and geranylgeranyl diphosphate (GGPP).

To further investigate the role of isoprenoids in steroidogenesis, other structurally related isoprenoids, such as geraniol (GOH) and farnesol (FOH) that have two and three isoprene units, respectively, as well as phytol (POH) and geranylgeranyl diphosphate (GGPP), were also examined (**Figure 6**). Accordingly, testosterone and progesterone levels were markedly increased upon treatment with POH and GGPP in I-10 cells. In contrast, FOH increased the levels of progesterone but not testosterone, whereas GOH did not affect steroidogenesis in I-10 cells [33]. These results indicate that most of the tested isoprenoids, and particularly POH, can stimulate the steroidogenic pathway in I-10 cells to the same extent as GGOH.

In summary, the novel role of MK-4 in stimulating steroidogenesis in I-10 cells through regulation of cAMP/PKA signaling may depend on GGOH and other structurally related isoprenoids. In addition, we found that MK-4, but not GGOH, enhanced glucose-stimulated insulin secretion (GSIS) by altering cAMP levels in INS-1 insulinoma cells (unpublished data). Some studies have reported that low testosterone levels could predict the development of type 2 diabetes and cardiovascular disease and have been linked to the increased risk of mortality in men [26–30]. It remains to be established if there is a direct connection between these diseases and vitamin K and what the different functions of MK-4 and GGOH may be. Taken together, these findings provide novel mechanistic insights in the process of steroidogenesis and GSIS and may be useful for the development of therapeutic strategies for men.

Author details

Hsin-Jung Ho, Hitoshi Shirakawa* and Michio Komai

*Address all correspondence to: shirakah@m.tohoku.ac.jp

Laboratory of Nutrition, Graduate School of Agricultural Science, Tohoku University, Sendai, Japan

References

[1] Davidson R.T., Foley A.L., Engelke J.A., Suttie J.W. Conversion of dietary phylloquinone to tissue menaquinone-4 in rats is not dependent on gut bacteria. J. Nutr. 1998;128(2):220–223.

[2] Thijssen H.H., Vervoort L.M., Schurgers L.J., Shearer M.J. Menadione is a metabolite of oral vitamin K. Br. J. Nutr. 2006;95(2):260–266.

[3] Nakagawa K., Hirota Y., Sawada N., Yuge N., Watanabe M., Uchino Y., et al. Identification of UBIAD1 as a novel human menaquinone-4 biosynthetic enzyme. Nature. 2010;468(7320):117–121. doi: 10.1038/nature09464.

[4] Shiraki M., Shiraki Y., Aoki C., Miura M. Vitamin K2 (menatetrenone) effectively prevents fractures and sustains lumbar bone mineral density in osteoporosis. Bone Miner. Res. 2000;15(3):515–521.

[5] Ohsaki Y., Shirakawa H., Hiwatashi K., Furukawa Y., Mizutani T., Komai M. Vitamin K suppresses lipopolysaccharide-induced inflammation in the rat. Biosci. Biotechnol. Biochem. 2006;70(4):926–932.

[6] Ohsaki Y., Shirakawa H., Miura A., Giriwono P.E., Sato S., Ohashi A., et al. Vitamin K suppresses the lipopolysaccharide-induced expression of inflammatory cytokines in cultured macrophage-like cells via the inhibition of the activation of nuclear factor kB through the repression of IKKα/β phosphorylation. J. Nutr. Biochem. 2010;21(11):1120–1126. doi: 10.1016/j.jnutbio.2009.09.011.

[7] Otsuka M., Kato N., Shao R.X., Hoshida Y., Ijichi H., Koike Y., et al. Vitamin K2 inhibits the growth and invasiveness of hepatocellular carcinoma cells via protein kinase A activation. Hepatology. 2004;40(1):243–251.

[8] Tokita H., Tsuchida A., Miyazawa K., Ohyashiki K., Katayanagi S., Sudo H., et al. Vitamin K2-induced antitumor effects via cell-cycle arrest and apoptosis in gastric cancer cell lines. Int. J. Mol. Med. 2006;17(2):235–243.

[9] Yokoyama T., Miyazawa K., Yoshida T., Ohyashiki K. Combination of vitamin K2 plus imatinib mesylate enhances induction of apoptosis in small cell lung cancer cell lines. Int. J. Oncol. 2005;1(26):33–40.

[10] Tabb M.M., Sun A., Zhou C., Grün F., Errandi J., Romero K., et al. Vitamin K2 regulation of bone homeostasis is mediated by the steroid and xenobiotic receptor SXR. J. Biol. Chem. 2003;278(4):43919–43927.

[11] Ichikawa T., Horie-Inoue K., Ikeda K., Blumberg B., Inoue S. Steroid and xenobiotic receptor SXR mediates vitamin K2-activated transcription of extracellular matrix-related genes and collagen accumulation in osteoblastic cells. J. Biol. Chem. 2006; 281(25):16927–16934.

[12] MacCorquodale D.W., Cheney L.C., Binkley S.B., Holcomb W.F., McKee R.W., Thayer Sidney A., et al. The constitution and synthesis of vitamin K1. J. Biol. Chem. 1939;131:357–370.

[13] Binkley S.B., McKee R.W., Thayer Sidney A., Doisy Edward A. The constitution of vitamin K2. J. Biol. Chem. 1940;133:721–729.

[14] Shirakawa H., Katsurai T., Komai M. Conversion of menaquinone-4 in animal organs and it functions. Jpn. Oil Chem. Soc. 2014;14(12):547–553.

[15] Huber A.M., Davidson K.W., O'Brien-Morse M.E., Sadowski J.A. Tissue phylloquinone and menaquinones in rats are affected by age and gender. J. Nutr. 1999;129(5):1039–1044.

[16] Payne A.H., Hardy M.P., editors. The Leydig Cell in Health and Disease. first ed. New Jersey: Human Press; 2007. 476 p. doi: 10.1007/978-1-59745–453-7.

[17] Shores M.M., Matsumoto A.M., Sloan K.L., Kivlahan D.R. Low serum testosterone and mortality in male veterans. Arch. Intern. Med. 2006;166(15):1660–1665.

[18] Huhtaniemi I. Late-onset hypogonadism: current concepts and controversies of pathogenesis, diagnosis and treatment. Asian J. Androl. 2014;16(2):192–202. doi: 10.4103/1008-682X.122336.

[19] Akishita M., Hashimoto M., Ohike Y., Ogawa S., Iijima K., Eto M., Ouchi Y. Low testosterone level as a predictor of cardiovascular events in Japanese men with coronary risk factors. Atherosclerosis. 2010;210(1):232–236. doi: 10.1016/j.atherosclerosis. 2009.10.037.

[20] Morris P.D., Channer K.S. Testosterone and cardiovascular disease in men. Asian J. Androl. 2012;14(3):428–435. doi: 10.1038/aja.2012.21.

[21] Beatrice A.M., Dutta D., Kumar M., Kumbenahalli Siddegowda S., Sinha A., Ray S., et al. Testosterone levels and type 2 diabetes in men: current knowledge and clinical implications. Diabetes Metab. Syndr. Obes. 2014;7:481–486. doi: 10.2147/DMSO.S50777.

[22] Tsang C.K., Kamei Y. Novel effect of vitamin K(1) (phylloquinone) and vitamin K(2) (menaquinone) on promoting nerve growth factor-mediated neurite outgrowth from PC12D cells. Neurosci. Lett. 2002;323(1):9–12.

[23] Otsuka M., Kato N., Shao R.X., Hoshida Y., Ijichi H., Koike Y., et al. Vitamin K2 inhibits the growth and invasiveness of hepatocellular carcinoma cells via protein kinase A activation. Hepatology. 2004;40(1):243–251.

[24] Ichikawa T., Horie-Inoue K., Ikeda K., Blumberg B., Inoue S. Vitamin K2 induces phosphorylation of protein kinase A and expression of novel target genes in osteoblastic cells. J. Mol. Endocrinol. 2007;39(4):239–247.

[25] Shirakawa H., Ohsaki Y., Minegishi Y., Takumi N., Ohinata K., Furukawa Y., et al. Vitamin K deficiency reduces testosterone production in the testis through down-

regulation of the Cyp11a a cholesterol side chain cleavage enzyme in rats. Biochim. Biophys. Acta 2006;1760(10):1482–1488.

[26] Ito A., Shirakawa H., Takumi N., Minegishi Y., Ohashi A., Howlader Z.H., et al. Menaquinone-4 enhances testosterone production in rats and testis-derived tumor cells. Lipids Health Dis. 2011;10:158.

[27] Takumi N., Shirakawa H., Ohsaki Y., Ito A., Watanabe T., Giriwono P.E., et al. Dietary vitamin K alleviates the reduction in testosterone production induced by lipopolysaccharide administration in rat testis. Food Funct. 2011;2(7):406–411. doi: 10.1039/c1fo10058k.

[28] Suhara Y., Hirota Y., Nakagawa K., Kamao M., Tsugawa N., Okano T. Design and synthesis of biologically active analogues of vitamin K2: evaluation of their biological activities with cultured human cell lines. Bioorg. Med. Chem. 2008;16(6):3108–3117.

[29] Fernandes N.V., Yeganehjoo H., Katuru R., DeBose-Boyd R.A., Morris L.L., Michon R. Geranylgeraniol suppresses the viability of human DU145 prostate carcinoma cells and the level of HMG CoA reductase. Exp. Biol. Med. (Maywood). 2013;238(11):1265–1274.

[30] Ohizumi H., Masuda Y., Nakajo S., Sakai I., Ohsawa S., Nakaya K. Geranylgeraniol is a potent inducer of apoptosis in tumor cells. J. Biochem. 1995;117(1):11–13.

[31] Masuda Y., Maeda S., Watanabe A., Sano Y., Aiuchi T., Nakajo S., et al. A novel 21-kDa cytochrome c-releasing factor is generated upon treatment of human leukemia U937 cells with geranylgeraniol. Biochem. Biophys. Res. Commun. 2006;346(2):454–460.

[32] Giriwono P.E., Shirakawa H., Ohsaki Y., Hata S., Kuriyama H., Sato S., et al. Dietary supplementation with geranylgeraniol suppresses lipopolysaccharide-induced inflammation via inhibition of nuclear factor-kB activation in rats. Eur. J. Nutr. 2013;52(3):1191–1199. doi: 10.1007/s00394-012-0429-y.

[33] Ho H.J., Shirakawa H., Yoshida R., Ito A., Maeda M., Goto T., et al. Geranylgeraniol enhances testosterone production via the cAMP/protein kinase A pathway in testis-derived I-10 tumor cells. Biosci. Biotechnol. Biochem. 2016;80(4):691-697. doi: 10.1080/09168451.2015.1123612.