Chapter 11

Vitamin K2 Facilitating Inter-Organ Cross-Talk

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67153

Abstract

This chapter features how vitamin K2 is instrumental in bringing about inter-organ communication, thus facilitating (a) a synthesis/secretion of the endocrine, humoral factors from various organs and (b) physiological responses to the said factors by a multitude of organ systems of the body, thus creating a 'lattice' of reciprocal regulatory loops in order to ensure endocrine homeostasis.

Keywords: vitamin K2, MK-4, MK-7, PXR/SXR, FoxO and FoxA families of transcription factors, PI3K/Akt cascade, endocrine homeostasis, deiodinase (DIO_2), NF-κB, interleukins, IGFs, MMPs, FGFs, irisin, osteonectin, UCP1, VEGF, GIP/GLP

1. Introduction

Recently, an abstract entitled 'Epigenetic factors involved in musculo-skeletal interaction - How skeletal muscle cells and osteoblasts derived from stem cells communicate with special reference to histone deacetylases (HDACS), transcription factors (TFs), microRNAs, and vitamin K2' was presented at an OMICS conference in Chicago, USA [1].

This work shows how the interactive axis consisting of 'Epigenator-Initiator-Maintainer' components determines the ultimate phenotypes of the cellular functions or the chain of reactions within different body organs and tissues. The initiator signals (i.e. histone modifications, phenotype modifications through transcriptional control, via microRNA species) constitute forces, slowly tilting the cell phenotype towards a more or less stable profile.

Despite this tendency, phenotypic characteristics could be subjected to alterations, that is, either weakened or reinforced, or even altered, resulting from underlying or developing diseases and/or gene therapy. The present work encompasses some data showing manipulations

of HDACs, transcription factors (TFs), as well as microRNAs and the impact of vitamin K2 (MK-7) on mineralizing cells (osteoblasts) and striated muscle cells exposed to either normal growth conditions or mediators of inflammation (i.e. Th-cells, macrophages, or interleukins), indicating that it is possible to engineer cells displaying an adapted phenotype where: (a) towards mineralization is reinforced, (b) untoward mineral deposition is halted and finally (c) mutual musculoskeletal interactions are 'reinforced'.

With the aid of various algorithms, one may reveal regulatory loops involving both TFs and microRNAs. The subjects TFs and microRNA species appeared to be part of an intricate hierarchical structure encompassing several classes of HDACs, including the Sirtuins, known to respond to cellular energy status (i.e. NADH/NAD^+ ratios). Finally, it was demonstrated that vitamin K2 (MK-7, via binding to the transcription factor SXR) interfered with a plethora of signalling pathways (such as the FoxA and FoxO families of transcription factors), the downstream of the signalling mechanisms represented by the PI3-kinase system (i.e. Akt/PKB and SGK, respectively), thus potentiating the cross-talk signals or suppressing the mineralizing character. It was concluded that vitamin K2 plays a pivotal role by optimizing the endocrine interaction between osteoblasts and striated muscle cells, facilitating a 'win-win' situation. Furthermore, we have shown that vitamin K2 may confer the ability for cross-talk between striated muscle cells and bones to include cells, such as insulin-producing β-cells, thyroid follicular cells, PTH-producing parathyroid cells and hepatocytes, in the absence or presence of inflammatory cells or their secreted cytokines/interleukins ± TNFα.

Sarcopenia (reduced muscle mass and/or function) and osteoporosis (bone brittleness) have generally been known for their relations to the locomotive syndrome and are linked to old age. Contrastingly, an increased muscle mass correlates with an enhanced bone mass and thus with a reduced fracture incidence. Genetic, as well as endocrine and mechanical factors, inflammation and nutritional states concurrently impinge on muscle tissue and bone metabolism.

Furthermore, a plethora of genes like myostatin and α-actinin-3 associate with both conditions. Factors such as vitamin D, growth hormones (like GH and IGF-1) and testosterone and pathological conditions with excess cortisol, as well as type I diabetes (T1DM), affect both muscle and bone tissues. It was shown that the genes Tmem119, osteoglycin and FAM5C may be critical for the commitment of myoprogenitor cells to the osteoblast lineage. Furthermore, osteoglycin and FAM5C might serve as muscle-derived humoral osteogenic factors. Others, encompassing myostatin, osteonectin, as well as IGF-1, irisin and osteocalcin, may also be associated with reciprocal metabolic interactions between muscle and bone [2].

2. Genetic factors and muscle/bone phenotypes

Genes such as myostatin, α-actinin-3, proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and myocyte enhancer factor 2C (MEF-2C) are included in GWAS (genome-wide association study) as believed to be involved in a concurrent loss of muscle and bone tissue [3]. Myostatin, on the other hand, has been shown to be a negative regulator of muscle mass. Alpha-actinin-3 has been demonstrated to be abundantly expressed in fast-twitch skeletal muscle fibres and may also affect their differentiation towards fast-twitch fibres. Finally,

http://dx.doi.org/10.5772/67153

it was shown [4] that a lack of α-actinin-3 may lead to a reduction in bone mineral density (BMD) in both humans and rodents.

PGC-1α seems to be instrumental in the modulation of mitochondrial biogenesis [5], and a further study established that PGC-1α elicited by physical activity seems to be crucial for oxidative metabolism in skeletal muscle fibres [6]. Furthermore, it was demonstrated that mitochondrial biogenesis induced by an enhancement in PGC-1α levels facilitates Wnt-mediated induction of osteoblastic differentiation of mesenchymal C3H10T1/2 cells [7]. These findings indicate that PGC-1α serves as a 'commitment' factor or inductor of stem cells to produce osteoblastic cells. Another essential factor is MEF-2C, which interacts with other myogenic regulatory factors, like Myf5 and MyoD, which in a synergistic fashion activates specific muscle-phenotypic genes. Animals devoid of MEF-2C in osteocytes make less sclerostin, a humoral factor acting as an inhibitor of the Wnt family of signalling molecules involved in osteoblast differentiation and bone formation. Thus, the MEF-2C-sclerostin signalling inhibits the formation of excessive bone mass and a 'healthy' turnover, which normally ensures minimal bone brittleness.

Qiu et al. [8] demonstrates that NF-κb-mediated signalling modulates myostatin transcription in myoblasts during cirrhosis-induced hyperammonaemia. This suggests that NF-κB antagonists are useful to reverse cirrhosis induced by sarcopenia. This observation also indicates that vitamin K2-induced modulation of NF-κB may determine the levels of humoral factors, which reciprocally regulate muscle and bone physiology. We have found [Gordeladze et al., 2015, unpublished] that preadipocytes, with mutated and superactive Gsα-induced adenylate cyclase activity, in the presence of vitamin K2 (MK-7), produce more beige-like adipocytes (see **Figure 1**) than large white adipocytes (ref), with an enhancement in PGC-1α levels (ref). Hence, it may be asserted that vitamin K2 facilitates Wnt-mediated induction of osteoblastic differentiation by

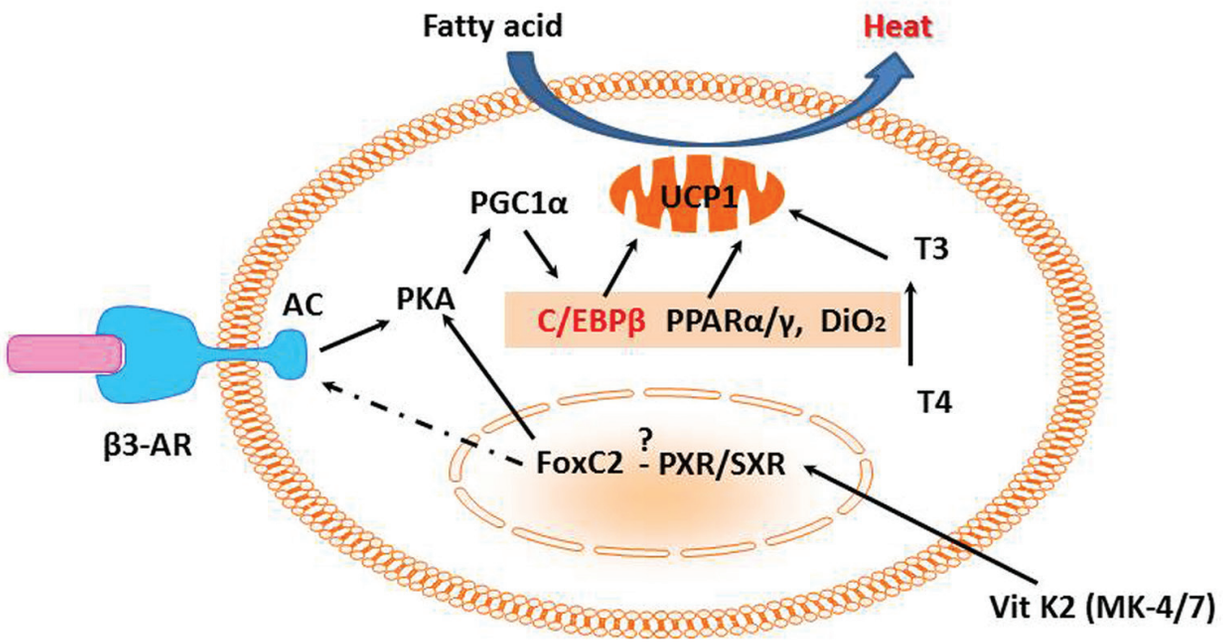

Figure 1. Putative working model showing how vitamin K2 may affect the hormonal signalling systems and transcription factors responsible for the transition of «white» adipocytes to «beige» adipocytes, thus blocking fat deposition and enhancing the production of heat from fatty acids.

enhancing the β-adrenoceptor and PKA-mediated signalling through PGC-1α of mesenchymal cells/stem cells, in order to fortify metabolic mechanisms 'ruled' by c/EBPβ, PPARα/γ and DiO_2.

However, the interaction between striated muscle cells and bones is illustrated in a better way in **Figure 2**. Here, it is shown that striated muscle cells communicate with 'the environment', consisting of other organs, such as white adipose tissue, liver, pancreas and bones through a multitude of endocrine/hormonal factors (see **Figure 2**(left)). However, if we just look closer at the reciprocal interactions between striated muscle cells and bone cells (osteoblasts, osteocytes and osteoclasts), there are still a large number of humoral factors, such as IGF-1, myostatin, osteoglycin, FAM5C, Irisin, Osteonectin, FGF2, IL-6, IL-7, IL-15, MMP-2, Sclerostin, Osteocalcin, MGP, VEGF and HGF (see **Figure 2**(right)), which 'capture' the two organs in a reciprocal regulatory 'looping system'. We have recently shown, for most part, the 'cross-talk' exchanged between these organs, particularly within the muscle-bone axis.

Vitamin K2 serves as a 'coupling agent', fine-tuning muscle-bone interactions, while concomitantly preserving its precision and strengthening it in the presence of inflammatory interleukins and INFα, and/or Th-1 and Th-17 cells.

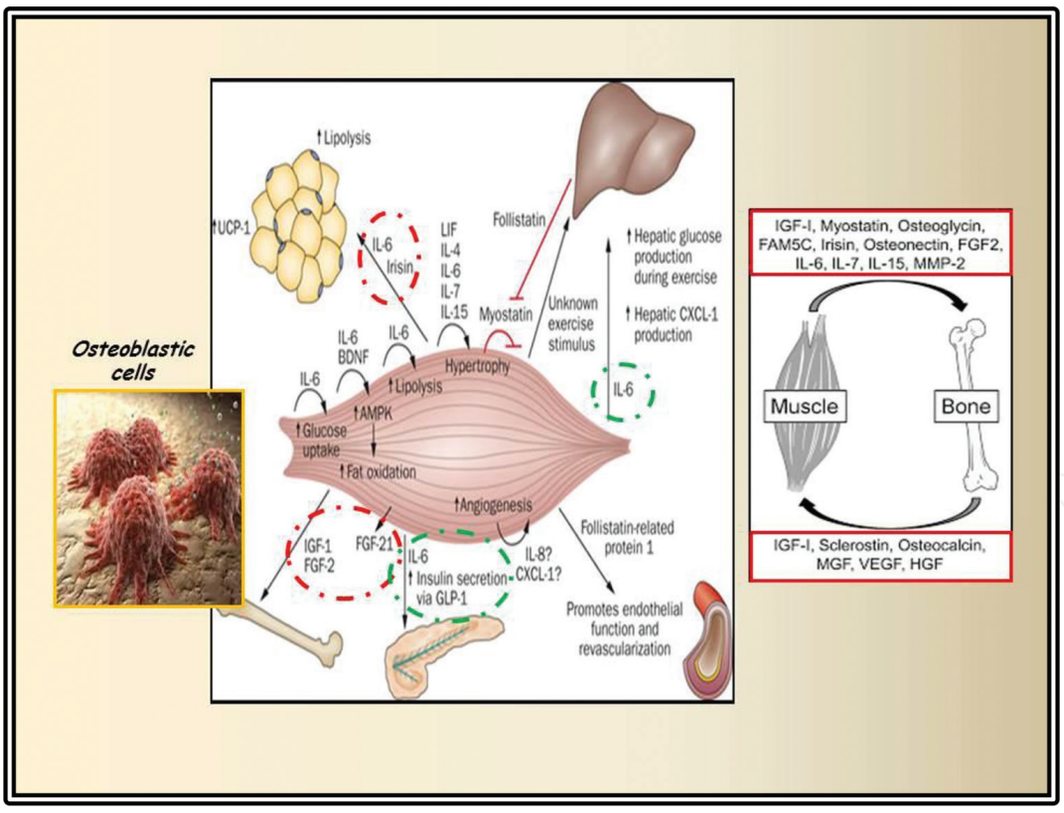

Figure 2. Endocrine communication between striated muscle cells and other organ systems, such as white adipose tissue, liver, bone and pancreas. Pay special attention to the plethora of hormones/cytokines being exchanged between the muscle and the bone. Ref.: Left: Benatti, F. B. & Pedersen, B. K. Nat. Rev. Rheumatol. 11, 86–97 (2015); published online 25 November 2014; doi:10.1038/nrrheum.2014.193. Right: Kawao N, Kaji H. J Cell Biochem. 2015 May;116(5):687–95. doi: 10.1002/jcb.25040. PMID: 25521430.

http://dx.doi.org/10.5772/67153

Since most of our cells throughout the body express the transcription factor PXR/SXR, binding vitamin K2, it may be asserted that vitamin K2 not only affects the phenotype of muscle and bone cells, shown here to interact in a reciprocal endocrine fashion but also (a) plays a major role in the determination and stabilization of the phenotype of a plethora of specialized cells in our body and (b) plays a pivotal role in the reciprocal interaction of various organ systems in our body to ensure optimal organ functions ('inter-organ cross-talk'). An interesting and elegant article written by Lara Pizzorno (see reference on last page) underscores the different effects of vitamin K2 in a comprehensive manner, supporting the notion that vitamin K2 is an essential biological factor supporting disease-free old age, which may be construed as if vitamin K2 is one important alimentary ingredient ensuring 'longevity'.

Longevity requires, of course, optimized and 'healthy' organ functioning throughout the body. Therefore, it is mandatory for the different organs of the body to communicate with each other and together form a 'cyn-organic' lattice where each organ communicates with the most part or all the others. Vitamin K2 may be one factor contributing to this inter-organ 'cross-talk', and there are several ways this little molecule exerts its integrative power. In this respect, the present book's chapter entitled 'Vitamin K2—small molecule with a large biological impact' featuring the molecular mechanisms, by which K2 exerts its actions, describes in detail how $DIO_{1,2,3}$ impacts the regulation of cell proliferation, lipogenesis, lipolysis, cholesterol metabolism, carbohydrate metabolism, muscle contraction, thermogenesis, cell communication, exocytosis, cell cycle regulation and growth regulation. Of particular interest is the type 2 deiodinase,(DIO_2), which, via higher brain centres, the pancreas, striated muscle cells, the liver as well as white and brown adipose tissues, converts T4 to its active form, T3, ensuring an integrated metabolic and hormonal homeostasis and 'steady state' or endocrine equilibrium between the different organ systems of the body. In this context, we have shown (ref) that vitamin K2 is able to sustain the cell phenotypes of different organ systems, such as bones, striated muscles and others in the presence of sub-chronical inflammation, as induced by the presence of either Th-1 cells, Th-17 cells, as well as a mixture of TNFα or inflammatory interleukins (e.g. IL-1 and IL-17).

Others have more directly proven that vitamin K2, via binding to PXR, affects both triglyceride turnover and gluconeogenesis in the liver (ref). The authors of this chapter describe how the MK-PXR complex via CD36, CPTA1 and SCD1 stimulates ketogenesis and hampers triglyceride production. Furthermore, they also show how the MK-PXR complex, via a cluster of transcription factors (FoxO1, CREB, PGC-1α and HNF4), stimulates the enzymes PEPCK1 and G6Pase in order to facilitate the metabolic conversion of lactate and amino acids to glucose (i.e. gluconeogenesis). This mechanism is remarkably like the one sketched in **Figure 1**, only that there are other members of the Fox family of transcription factors involved!

3. Novel findings related to the biology of vitamin K2

A summary of a literature survey, extracting articles from PubMed, featuring new research on the biological impact of vitamin K2 published in 2015 and 2016, gave the following results:

a. Shetty A et al. Urol Oncol. 2016 Sep 27. pii: S1078-1439(16)30108-9. doi: 10.1016/j.urolonc.2016.05.027. **Hepatoma-derived growth factor: A survival-related protein in prostate oncogenesis and a potential target for vitamin K2**.

Excerpt from the article: Hepatoma-derived growth factor (HDGF) is correlated with a poor prognosis in cancers. Forced overexpression of HDGF sustained viability of RWPE-1 human prostate cancer cells, while a knockdown of HDGF hampered their proliferation. Furthermore, using a HDGF antibody along with vitamin K2 reduced cell proliferation and inhibited NF-κB expression.

b. Sanguineti R et al. J Biol Regul Homeost Agents. 2016 Jul-Sep;30(3):713–726. **Vitamins D3 and K2 may partially counterbalance the detrimental effects of pentosidine in ex vivo human osteoblasts**.

Excerpt from the article: Osteoporosis is a multifaceted metabolic disorder, construed as inadequate bone strength. It was recently demonstrated that advanced glycation end products (AGEs) (e.g. pentosidine = PENT) may serve as indicators of senile bone brittleness. Optimal responses to hormones, like 1,25-dihydroxyvitamin D_3, serve as prerequisites for optimal, time-dependent osteoblast functioning (the oscillation between bone building and bone resorption phases). Vitamin K2 (MK-4/7) may enhance net vitamin D-induced bone formation.

Ex vivo human osteoblasts, incubated with vitamins D_3 and K2 and exposed to PENT for 72 hours, were assessed for gene expression, that is, ALP, COLIα1 and osteocalcin, as well as the RANKL/OPG ratio to assess net bone formation (as a result of the continuous remodelling process). The expression of RAGE, which is a well-characterized receptor of advanced glycation end products (AGEs), was also monitored. PENT + vitamins significantly inhibited ALP expression/secretion but did not show any impact on gene expression, which may be construed as hampered functional osteoblastic activity. Furthermore, PENT + vitamins enhanced gene expression of collagen, while protein secretion remained unchanged. Intracellular levels of collagen were partially lowered, while a fall in BGP gene expression, as well as intracellular protein levels, was seen with PENT exposure. The ratio RANKL/OPG was augmented, favouring bone resorption. Expression of the RAGE gene was, however, lowered, indicating that the detrimental AGE accumulation in the bone was attenuated and/or counteracted by the vitamins' D_3 and K2 exposure.

c. Ronn SH et al. Eur J Endocrinol. 2016 Sep 13. pii: EJE-16-0498. [Epub ahead of print] PLoS One. 2016 Aug 29;11(8):e0161886. doi: 10.1371/journal.pone.0161886. eCollection 2016. **Vitamin K2 (menaquinone-7) prevents age-related deterioration of trabecular bone microarchitecture at the tibia in postmenopausal women**.

Excerpt from the article: Vitamin K2 allegedly protects one against bone loss and fractures; however, its effect on bone quality has hitherto not been investigated. This article explores the effect of MK-7 on undercarboxylated osteocalcin (ucOC), bone mass, as well as bone quality. A randomized, 1-year, placebo-controlled, double-blind clinical trial was conducted, where the effect of some 400 μg of MK-7 (including supplementation with calcium and vitamin D) was assessed.

UcOC was diminished in the MK-7 group as compared with the placebo group subsequent to 3 months of treatment. Furthermore, the trabecular number in tibia was unchanged in the MK-7

group, while lowered in the placebo group. The trabecular spacing remained unchanged in the MK-7 group, while it increased in the placebo group. Finally, trabecular thickness stayed unchanged in the MK-7 group, while it increased in the placebo group. And one could not find significant differences between the groups at the radius or in BMD at any site.

The alterations in microarchitecture of bone tissue within the placebo group were in line with the age-related deteriorations of the trabecular bone structure, including trabecula loss, with a concomitant enhanced average thickness of the ones remaining. This may indicate that MK-7 sustains proper tibial trabecular bone microstructures.

d. Duan F et al. PLoS One. 2016 Aug 29;11(8):e0161886. doi: 10.1371/journal.pone. 0161886. eCollection 2016. **Vitamin K2 induces mitochondria-related apoptosis in human bladder cancer cells via ROS and JNK/p38 MAPK signal pathways**.

Excerpt from the article: Vitamin K2's impact on apoptosis in cancerous cells has been elucidated in many studies. In the present study, it was shown that vitamin K2 induced apoptosis in cancer cells of the bladder through mitochondrial pathways, that is (a) loss of mitochondrial membrane potential and (b) release of cytochrome C and the activation of the caspase-3 cascade. Additionally, c-Jun N-terminal kinase (JNK) and p38 MAPK phosphorylation were enhanced in vitamin K2-treated cells. The generation of reactive oxygen species (ROS) was detected in bladder cancer cells; however, the treatment with vitamin K2 and the antioxidant N-acetyl cysteine (NAC) blocked (a) the vitamin K2-triggered apoptosis, (b) the loss of mitochondria membrane potential and (c) the activation of JNK and p38 MAPK.

Hence, it seems that vitamin K2 brings about apoptosis in bladder cancer cells through: (a) ROS-mediated JNK/p38 MAPK and (b) mitochondrial pathways.

e. Yu YX et al. Acta Pharmacol Sin. 2016 Sep;37(9):1178–89. doi: 10.1038/aps. 2016.68. Epub 2016 Aug 8. **Vitamin K2 suppresses rotenone-induced microglial activation in vitro**.

Excerpt from the article: It has been shown that environmental factors (e.g. rotenone and others) cause neuroinflammation, contributing to the development of Parkinson's disease. Here, one explored molecular mechanism, pertaining to the repression by vitamin K2 (MK-4), on in vitro rotenone induced microglial activation.

The cell line (BV2) was exposed to rotenone in the presence or absence of MK-4. The levels of TNF-α or IL-1β were assessed, using ELISA technology. BV2 cells treated with rotenone with or without MK-4 were subjected to analyses of (a) the mitochondrial membrane potential, (b) ROS production, (c) immunofluorescence or (d) immunoblot assays. The neuroblastoma cells were exposed to conditioned media of BV2 cells, having been exposed to rotenone ± MK-4, and cell viability was assessed.

In rotenone-treated BV2 cells, MK-4 dose dependently counteracted upregulation of iNOS and COX-2 expression in the cells, while also the production of TNF-α and IL-1β. MK-4 significantly blocked rotenone-induced translocation to the nucleus of NF-κB. MK-4 significantly also attenuated rotenone-provoked p38 activation, ROS production and caspase-1 activation.

Vitamin K2 can directly suppress rotenone-induced activation of microglial BV2 cells in vitro by repressing ROS production and p38 activation.

f. Vissers LE et al. Atherosclerosis. 2016 Sep;252:15–20. doi: 10.1016/j.atherosclerosis.2016.07.915. Epub 2016 Jul 25. **The relationship between vitamin K and peripheral arterial disease (PAD)**.

Excerpt from the article: Vitamin K1 (phylloquinone) and vitamin K2 (menaquinones) are believed to diminish the risk of cardiovascular diseases by reducing or blocking calcification of the vascular bed.

The association between the intake of vitamins K1 and K2 with PAD was analysed in a prospective cohort of some 36,500 participants. The occurrence of PAD was obtained from national registries. Baseline intakes of K1 and K2s were calculated using standard food-frequency questionnaires.

During 12 years of follow-up, some 500 cases of PAD were documented. Menaquinone (K2) intake was associated with a reduced risk of PAD. A stronger association was observed in participants suffering from either hypertension or diabetes, respectively. Phylloquinone (K1) intake was not associated with PAD risk.

g. Villa, JK et al. Crit Rev Food Sci Nutr. 2016 Jul 20:0. [Epub ahead of print]. **Effect of vitamin K in bone metabolism and vascular calcification: a review of mechanisms of action and evidences**.

Excerpt from the article: Osteoporosis is associated with a public health concern, featuring the enhanced risk of incurring fractured bones, as well as vascular calcification. The family of vitamin K represents unique benefits on these issues, even though its aspects are far from exhaustively studied. The two important forms of vitamin K are phylloquinone = vitamin K1 and menaquinone = vitamin K2 (MK-4 and MK-7). In the present work, we investigated, in particular, the effect of vitamin K2 in bones and blood vessels. In addition to the known effects of vitamin K2, this particular form has been demonstrated to support bone formation via stimulating osteoblast differentiation, as well as osteocalcin carboxylation and enhanced ALP activity, IGF-1, as well as other growth-promoting protein (DF-15 and STCal-2) levels.

Furthermore, vitamin K2 lowers the osteoblast levels of proapoptotic proteins like Fas and Bax, while also hampering osteoclast differentiation via enhanced levels of osteoprotegerin (OPG) and reducing the amount of 'receptor activator of nuclear factor kappa-B ligand' NF-κB. In blood vessels, vitamin K2 diminishes the production of hydroxyapatite by carboxylating matrix-Gla protein, as well as the Gla-rich protein. Vitamin K2 hampers the death (apoptosis) of smooth vascular muscle cells, as well as reducing phenotype alteration of vascular smooth muscle cells to osteoblastic cells. The standard dosage of vitamin K2 studies of man amounts to 45 mg/day, which allows beneficial effects on bone and vasculature, especially in post-menopausal women with osteoporosis.

h. Ochsner et al., Endocrinol. 2016 Aug;30(8):937–48. doi: 10.1210/me.2016-1095. Epub 2016 Jul 13. **A reference transcriptome for constitutive androstane receptor and pregnane X receptor xenobiotic signaling**.

Excerpt from the article: PXR = PXR/NR1I3 and constitutive androstane receptor (CAR = CAR/NR1I2) both belong to the nuclear receptor (NR) superfamily of ligand-regulated TF2.

They are both mediators of endocrine-disrupting chemical signalling. In this work a "reference transcriptome" was generated, a 'reference transcriptome' encompassing members of frequently and differentially expressed of genes across some 160 experiments compiled from some 20 datasets, describing perturbations of both CAR- and PXR-based signalling pathways. Omitting the genes encoding members of the xenobiotic 'stress response', the ranking of genes especially involved in the metabolism of carbohydrate sheds light on the role of xenobiotics and thus vitamin K2, in the metabolic syndrome.

i. Zhang Y et al. Int J Biol Sci. 2016 Apr 28;12(7):776–85. doi: 10.7150/ijbs.15248. eCollection 2016. **Vitamin K2 ameliorates damage of blood vessels by glucocorticoid: a potential mechanism for its protective effects in glucocorticoid-induced osteonecrosis of the femoral head in a rat model**.

Excerpt from the article: Glucocorticoids have been reported to lower the blood vessels' number and also lower the blood supply in the femoral head. This is known to serve as an important mechanism, by which glucocorticoids induce osteonecrosis of the femoral head (ONFH). In order to prevent manifest drug-induced ONFH, bone formation with concomitant angiogenesis would be a beneficial treatment.

The present study investigates whether vitamin K2 could stimulate the formation of new blood vessels in the presence of glucocorticoids, both in vitro and in vivo. The effect of vitamin K2 on parameters, such as viability, migration, in vitro tube formation and key genes (like VEGF and PDGFB) incubated with or without dexamethasone were elucidated. VEGF, TGF-β and BMP-2, angiogenesis-related proteins secreted by osteoblastic cells (MG63 cell batches), were also detected.

Additionally, blood vessels in the femoral head of rats (given with or without a glucocorticoid and vitamin K2) were assessed. It showed that vitamin K2 fully protected the endothelial cells used from apoptosis, induced by dexamethasone. Furthermore, endothelial cell migration and in vitro tube formation were promoted. Furthermore, angiogenesis-related proteins were also stimulated by vitamin K2. In vivo studies revealed enhanced blood vessel volume with CD31-positive cells in rats, co-treated with vitamin K2.

In general, vitamin K2 demonstrated an ability to promote and sustain angiogenesis *in vitro* and to ameliorate/rescue vessels of the femoral head in glucocorticoid-treated rats *in vivo*. This indicates that vitamin K2 holds a role as a promising drug to be used in the prevention of steroid-induced ONFH.

j. Mi et al., Int J Exp Pathol. 2016 Apr;97(2):187–93. doi: 10.1111/iep.12178. Epub 2016 Jun 3**. Establishing a rat model for the study of vitamin K deficiency**.

Excerpt from the article: The main vitamin K-deficient model (i.e. the minidose warfarin exposure) differs from the pathological model of vitamin K deficiency, being a shortage of vitamin K. The present work aimed to establish a new method to provoke vitamin K deficiency in rats by combining vitamin K-deficient diets with intragastrical administration of gentamicin. In the diet- and gentamicin-provoked vitamin K-deficient animals, all rats suffered hepatic vitamin K1 and K2 loss and consequently an extended period of APTT. And, within the 21-day

treatment group, one could also measure prolonged PT, as well as the decreasing FIX activities. Furthermore, in the 28-day treatment group, unmeasurable vitamin K1 and K2, enhanced PT and APTT values, as well as a decrease in FII, FVII, FIX and FX activities sustained the hypothesis that serious vitamin K deficiencies in the experimental animals had taken place. It was therefore hypothesized that the diet- and gentamicin-induced vitamin K-deficient model system could be used to study vitamin K's status. This vitamin K-deficient 28-day model could therefore be applied to research related to both the status of vitamin K and vitamin K-dependent coagulation per se. Hence, it was asserted that the combination of a vitamin K-deficient diet with the administration of gentamicin yields an ideal model to study vitamin K deficiency.

k. Okuyama et al. 2016;98(3–4):134–70. doi: 10.1159/000446704. Epub 2016 Jun 2. **Medicines and vegetable oils as hidden causes of cardiovascular disease and diabetes**.

Excerpt from the article: A coupling has been observed between cardiovascular diseases (CVD) and diabetes mellitus type 2 (T2DM); however, a causal relationship has hitherto not been made. Irrespective of aetiology, cholesterol-lowering medication (statins) has been recommended for both patient categories.

Statin-induced suppression of prenyl moieties in the pathway of cholesterol biosynthesis has been associated with both atherosclerosis and heart failure. However, both vegetable and hydrogenated oils appear to shorten the survival period incurred by stroke-prone spontaneously hypertensive animals through (a) downregulated platelet number, (b) enhanced haemorrhagic incidences and (c) aberrant kidney functioning. These phenomena are not related to their molecular constituents, and the present oils and drugs (i.e. statins and warfarin) share common mechanisms blocking vitamin K2-dependent biochemical processes.

l. Zhou C. Biochim Biophys Acta. 2016 Sep;1859(9):1112–20. doi: 10.1016/j.bbagrm.2016.02.015. Epub 2016 Feb 26. **Novel functions of PXR in cardiometabolic disease**.

Excerpt from article: Cardiometabolic disease is a worldwide epidemic, and our chemical environment is rapidly changing to the worse, resulting in an enhanced frequency and morbidity of chronic human diseases. But, mechanisms of how exposure to chemical compounds brings about cardiometabolic ailments are not well understood. A plethora of chemicals have, however, been shown to serve as activators of the pregnane X receptor (PXR), which, apart from serving as a nuclear receptor functioning as a xenobiotic sensor, also binds vitamin K2.

Recent investigations have unravelled new and intriguing roles of PXR in modulating potentially debilitating conditions such as obesity, insulin sensitivity, lipid homeostasis, atherogenesis and vascular functioning. The present reports indicate that PXR signalling significantly impacts pathophysiological conditions like cardiometabolic diseases in humans. This discovery of 'new' effects of PXR in the mentioned disease complexes not only heavily contributes to our comprehension of 'gene versus environment interactions', predisposing man to chronic disabilities, but also sheds light on future treatment modalities and the importance of optimal intake of vitamin K2, which binds to PXR, enabling a plethora of tissues in the body to function properly under strain from extrinsic and intrinsic challenges. The present article is part of a special issue called 'Xenobiotic nuclear receptors: New Tricks for An Old Dog'. Editor: Dr. Wen Xie.

m. Liu et al. PLoS One. 2016 Feb 18;11(2):e0149639.doi: 10.1371/journal.pone. 0149639. eCollection 2016. **Role of UBIAD1 in intracellular cholesterol metabolism and vascular cell calcification**.

Excerpt from an article: Vascular calcification is a risk factor coupled to mortality suffering from chronic kidney disease. Cholesterol turnover is linked to this vascular calcification process. In the present work, the role of the UbiA prenyltransferase domain containing 1 (UBIAD1) in cholesterol turnover and vascular cell sclerosis (i.e. calcification) was scrutinized.

Human umbilical vein smooth muscle cells (HUVSMCs) were exposed to (a) a traditional growth medium (with 1.4 mmol/L Pi) or (b) calcification medium (3.0 mmol/L Pi). When treated with medium (b), HUVSMCs were incubated with cholesterol or menaquinone-4 (a product synthesized by UBIAD1). Matrix calcium quantitation, alkaline phosphatase (ALP) levels and cellular cholesterol menaquinone-4 (vitamin K2) levels were analysed.

To make the story short, it was concluded that (a) high intracellular cholesterol content contributes to phosphate-induced vascular cell differentiation and calcification, (b) UBIAD1 or menaquinone-4 (vitamin K2) decreased vascular cell differentiation and calcification through its potent role of reciprocally modulating cellular cholesterol.

n. Litwa E. et al. J Steroid Biochem Mol Biol. 2016 Feb;156:43–52. doi: 10.1016/j.jsbmb. 2015.11.018. Epub 2015 Nov 28. **RXRα, PXR and CAR xenobiotic receptors mediate the apoptotic and neurotoxic actions of nonylphenol in mouse hippocampal cells**.

Excerpt from the article: Here, the authors investigated the role of RXR (the retinoid receptor), PXR and CAR (the constitutive androstane receptor) as to the apoptotic and toxic impacts of nonylphenol (NP) in neuronal mouse cell cultures. The present work showed that NP stimulated caspase-3, while inducing lactate dehydrogenase (LDH) activity in hippocampal cells, reactions which were paralleled by an enhancement of protein levels of RXRα, PXR as well as CAR. NP enhanced RXR, PXR and CAR mRNA synthesis, and the present effects preceded an enhancement in corresponding protein levels. The staining techniques applied here also indicated an NP-induced translocation of receptor-specific immunofluorescence from cytoplasm to the nucleus.

The use of specific siRNAs further indicated that RXR-, PXR- and CAR-siRNA-transfected cells had turned less vulnerable to NP-induced stimulation of caspase-3 and LDH, hence underscoring their involvement in RXRα/PXR/CAR, signalling pathways in the apoptotic-neurotoxic reactions induced by NP.

o. Sy C. et al. Mar Drugs. 2015 Nov 19;13(11):7020–39. doi: 10.3390/md13117020. **Interactions between carotenoids from marine bacteria and other micronutrients: impact on stability and antioxidant activity**.

Excerpt from the article: Spore forming and pigmented marine bacteria, like the Bacillus indicus HU36, serve as natural sources of oxygenated carotenoids. In the present study, the stability, as well as the antioxidant activity (i.e. resistance to lipid peroxidation), of HU36 carotenoids with bacterial MK-7 was investigated.

Unexpectedly, MK-7 substantially improved the ability of HU36 carotenoids to block Fe(II)-induced lipid peroxidation, even though MK-7 remained unconsumed in the incubation medium. Hence, it was asserted that their presence modifies the antioxidant properties exerted by the carotenoids, probably by allowing them to scavenge radicals. The HU36 carotenoids and phenol-derived antioxidants showed synergism in the inhibition of linoleic acid peroxidation. This reaction might have risen from antioxidants, which interacted via heme-iron-based electron transfer.

p. Puri A. et al. J Food Sci Technol. 2015 Dec;52(12):8228–35. doi: 10.1007/s13197-015-1903-3. Epub 2015 Jun 23. **Effect of sequential bio-processing conditions on the content and composition of vitamin K2 and isoflavones in fermented soy food**.

Excerpt from the article: The present paper features the effect of sequential addition of *Bifidobacterium bifidum*, *Bacillus subtilis*, as well as *Rhizopus oligosporus* on the contents and composition of vitamin K2 contained within fermented soya foods. Soya beans, fermented with *B. bifidum*, were treated as described: The fermented bacterial bulk mass was re-fermented with a co-culture of *B. subtilis* and *R. oligosporus*. This study indicated that the co-fermentation of soya beans with different microbe combinations, however, in a given, predefined sequence, may enhance nutritional value better than mono-culture fermentations, which is a result of the positive correlation between applied enzymes (lipase, phytase, β-glucosidase), menaquinone-7 (vitamin K2) and soya isoflavone contents.

q. Poon CC. et al. Eur J Pharmacol. 2015 Nov 15;767:30–40. doi: 10.1016/j.ejphar. 2015.09.048. Epub 2015 Oct 8. **In vitro vitamin K(2) and 1α,25-dihydroxyvitamin D(3) combination enhances osteoblasts anabolism of diabetic mice**.

Excerpt from the article: The present study features the anabolic effect and cellular action of vitamin K2 or/and 1α,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$) on iliac crest osteoblasts from C57BL/KsJ lean (+/+), as well as obese and diabetic (db/db) mice. A lower ALP (alkaline phosphatase) activity and a reduced expression of anabolic bone markers and osseous formation-related transcription factors (i.e. OC = osteocalcin, Dlx5, Runx2, ATF4 and OSX) were consistently observed in the osteoblastic cells of db/db mice, as compared with lean mice. Significantly higher deposits of Ca^{2+} by osteoblastic cells were seen in lean mice, as compared with db/db mice. Concomitant administration of vitamin K2 (MK-7) and active vitamin D (1,25$(OH)_2D_3$) brought about an increase in calcium deposits by osteoblasts in all animals. Furthermore, vitamins K2 and D successively (for 3 weeks) augmented levels of the anabolic bone turnover markers, including bone formation-related transcription factors. The combined treatment with vitamin K2 plus vitamin 1,25$(OH)_2D_3$ substantially stimulated migration, as well as the reappearance of both surface microvilli and ruffles expressed by osteoblasts of the db/db mice. Hence, the present data indicate that the vitamin K2 plus vitamin D_3 combination might serve as a novel therapeutic strategy for the treatment of diabetes-associated osteoporosis.

r. Qin XY. J Nutr Sci Vitaminol (Tokyo). 2015;61(4):285–90. doi: 10.3177/jnsv.61.285. **Carboxylic derivatives of vitamin K2 inhibit hepatocellular carcinoma cell growth through caspase/transglutaminase-related signaling pathways**.

Excerpt from the article: The chemoprevention of liver cancer (e.g. Hepatocellular carcinoma = HCC) has turned out to be one of the most challenging aspects of medical research. Vitamin K2 (MK-4, MK-7) has especially been launched for its established chemopreventive effect in the treatment of HCC, while inconsistent or contradicting results reported in clinical trials have emerged. The study described in the present paper was undertaken to add to our understanding of the anti-HCC cell proliferative effect of vitamin K2 and its derivatives, taking its chemical structure into account. However, no marked effects were observed with the original vitamin K2, while vitamin K2 derivatives, bearing isoprene units as well as a carboxyl-terminated side chains, appeared to inhibit the growth of HCC cells in a dose-dependent manner without damaging normal hepatocytes. Traditional loss-of-function analyses concluded beyond doubt that the anti-HCC cell effect witnessed by the vitamin K2 derivatives was not conferred by the vitamin K2 binding protein, 'Bcl-2 homologous antagonist/killer', known as 'Bak', but rather associated with the caspase-transglutaminase related pathway of signalling.

s. Hey H. Ugeskr Laeger. 2015 Aug 3;177(32):V12140700. **[Vitamin K2 influences several diseases]**. [Article in Danish]

Excerpt from the article: In the present paper, the evidence of the biological effects of vitamin K2 is discussed. Deficiency of vitamin K2 is indeed a factor in a plethora of chronic diseases, such as diabetes, osteoporosis, cancer, inflammations and cardiovascular ailments. Vitamin K2 deficiency is very commonly associated with the diseases mentioned above, even though vitamin K2 is rarely included in the treatment regimens used by clinicians. A multitude of randomized clinical investigations have demonstrated that patients with ailments, such as osteoporosis, cancer, cardiovascular diseases, Alzheimer's disease, adiposity and many others, can benefit from vitamin K2 supplementation. However, further studies are needed in order to ascertain the effect of vitamin K2 supplements in patients with diabetes and inflammatory bowel diseases.

As a last comment, the readers are highly recommended to read the article by Lara Pizzorno (http://weblisting.freetemplatespot.com/lmreview.com/), a compilation of beneficial features of vitamin K2 as: 'Essential for the Prevention of Age-Associated Chronic Diseases' amongst other articles on the subject of 'Longevity'.

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