# VITAMIN K2
## VITAL FOR HEALTH AND WELLBEING

**CHAPTER 5** The Impact of Vitamin K2
on Energy Metabolism
by Mona Møller, Serena Tonstad, Tone Bathen
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## Abstract

Environmental and behavioral adaptations introduced during the last decades have synergistically enhanced man's lifespan, but also paved the ground for disease states involving impairment of multiple organs, which are both modulating and depending on homeostatic calorie "accounting."

Diabetes, obesity, and/or bone brittleness now occur frequently in our society, inducing ailments affecting overall health and well-being. Therefore, an improved comprehension of how organs (e.g. bone and adipose tissue) may provide homeostasis and sound strategies to treat these diseases, thus improving health and life quality of most age categories, should be sought. The steroid and xenobiotic receptor (SXR) (pregnane X receptor = PXR) is a nuclear steroid-like hormone receptor, stimulated by hormones, steroids, drugs, and xenobiotic compounds. SXR exhibits a versatile ligand binding domain, serving as a xenobiotic sensor, regulating xenobiotic clearance from the liver and intestine. However, new and interesting functions of SXR in the regulation of inflammatory processes, cholecalciferol and bone metabolism, lipid and energy homeostasis, and cancer therapy, have emerged.

Hence, the discovery and pharmacological development of new PXR modulators, like vitamin K2, represent an interesting and innovative therapeutic approach to combat various diseases, of which glucose and lipid metabolism (i.e., energy metabolism and adiposity) should be emphasized.

**Keywords:** vitamin K2, energy metabolism, PXR, SXR

## 1. Introduction

In the past years, we have seen a plethora of research reports illuminating the link between bone physiology and energy metabolism, even though the central and sympathetic nervous

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systems, but also the gastrointestinal and pancreatic axes serve essential functions related to the systemic regulation of energy expenditure. It may be asserted that the fat tissue is more prominent due to its basic implication in storing and dissipating energy. During the last 25 years, a major progress has been communicated within the medical societies, featuring a modern understanding of the origin of fat tissues, their specialized characteristics and functioning, as well as the pathophysiological consequences of their impairment. These advances consequently lead to the discovery that adipose tissue metabolism is heavily coupled to the homeostasis of the skeleton.

Fat tissue is able to deposit and releases energy rich compounds during feeding and fasting, respectively, and it modulates the energy homeostasis in a versatility of organs via its endocrine potential. Fat cells (adipocytes) may accumulate energy in the form of triglycerides, as well as burning it by degrading fatty acids via so-called β-oxidation. Additionally, adipocytes produce and release so-called adipokines, among which leptin and adiponectin are the most important ones. These hormones regulate both the ingestion of calories, as well as the body's sensitivity to insulin. The functional multiplicity of adipose tissue is sustained by various subtypes of adipocytes in depot storing fat. Mitochondria-sparse white adipose tissue (WAT), characterized as visceral and subcutaneous fat, stores energy as triglycerides for which the level is regulated by the body's sensitivity to insulin and the overall metabolism of glucose in the liver and skeletal muscle cells, respectively. Contrastingly, mitochondria-enriched brown adipose tissue (BAT), which in adults is positioned as discrete entities localized in the neck and other regions of the trunk of the body [1], serves to dissipate energy to sustain adaptive thermogenesis [2]. This process is facilitated by the action of uncoupling protein 1 (UCP1), stimulating a leakage of protons, in order to uncouple respiration from ATP synthesis, thus favoring heat production. The thermogenesis sustained by BAT is mastered via the central nervous system, the impact of both the signaling systems comprised by catecholamines (i.e., β-adrenergic signaling), as well as deiodinase 2 (Dio2)-facilitated thyroid hormone conversion from T4 (thyroxine) to T3 (triiodothyronine). As an extra feature, compared to its role in adaptive thermogenesis, BAT is also protecting against obesity, as well as against insulin resistance and development of diabetes [5–8]. Finally, it is worth noticing that genetic ablation of BAT in small experimental animals is resulting in diet-induced obesity, diabetes mellitus with insulin resistance, as well as enhanced blood lipids [3].

It has been asserted that BAT may originate from two sources. The classical or ordinary, preformed BAT stems from Myf5-positive dermomyotomal progenitor cells which may also yield skin and muscle, as well as functioning in so-called nonshivering thermogenesis [4]. On the other hand, Myf5-negative progenitor cells may differentiate into white adipocytes which play a role in energy storage, or to BAT-like or "beige" fat cells. The latter adipocytes demonstrate both brown and white fat cell characteristics [5]. Of major importance is the fact that BAT-like adipocytes can be transformed into WAT-like adipocytes through a plethora of mechanisms, of which a few deserves mentioning; cold exposure, endocrine action of FGF21 [6] or irisin [7], and via transcriptional regulators including FoxC2 [8], PRDM16 [9], and PPARc [10], which lead to SirT1-mediated deacetylation of the PPARc protein [11]. Beige fat possesses powerful antiobesity and antidiabetic activities. An overexpression of BAT-specific transcription factors, i.e., either FoxC2 or PRDM16 in WAT adipocytes,

has been shown to protect mice from diet-induced obesity and metabolic dysfunction [9]. Furthermore, ablation of beige fat cells by adipocyte-only deletion of the transcriptional modulator PRDM16, yields experimental animals, which become prone to diet-induced obesity and ensuing insulin resistance [12].

In line with the cited article published by Lecka-Czernik et al. in Archives of Biochemistry and Biophysics, 2014 [13], we performed the following experiment: Human adipose stem cells and 3T3-L1 preadipocytes, the latter with an activated mutation (ref) in the $G_{i2\alpha}$-protein were differentiated into fully mature adipocytes as indicated by coloration with Alizarin Red. Interestingly, both adipocyte species accumulated triglycerides upon differentiation to mature adipocytes; however, exposure to vitamin K2 (MK-7) diminished their ability to turn into fully mature adipocytes. Furthermore, both adipocyte species were tested for expression of various genes differing white adipocytes from beige adipocytes. From the gene expression profile, featuring genes like the beta-adrenergic receptor β3-AR, Foxc2, PGC1α, PPARα, Dio2, UCP1, Adipoq, and Leptin, it was quite obvious that the preadipocytes in question differentiated more in the "direction" of beige, lipid (or fatty acid) metabolizing adipocytes than into white, triglyceride-storing adipocytes. Hence, it could be concluded or hypothesized that vitamin K2 (MK-7), in fact, was able to direct preadipocytes into becoming an energy-dissipating, rather than energy-storing adipocyte phenotype (see **Figure 1**, left and right panels). A putative model featuring this development, referring to a suggested mechanism, is given in **Figure 2**.

Here, we have demonstrated that vitamin K2 affects the differentiation of preadipocytes to mature adipocytes, ensuring that the "end-point" phenotype may be tilted in the direction of the beige, energy "dissipating" phenotype. In their paper from 2014, Lecka-Czernik and coworkers [13] assert that an impairment in fat function correlates with a reduced bone mass and an increased incidence of fractures. But, the question posed by the authors is: does accumulation of bone marrow adipose tissue (BMAT) exert a detrimental effect on bone structure and or mineralization, and will a diminished bone mass stimulate the accumulation of BMAT? Historically, BMAT was construed as a dormant or inert type of fat that accumulated within the bone marrow in order to pose as empty space filling subsequent to involution of hematopoietic tissue. Hence, its relationship with a lower bone mass was construed as circumstantial. However, novel evidence asserted that marrow adipogenesis should be construed as a process, which is tightly bound to the differentiation of osteoblasts, since they "share" common precursor cells, and they are subjected to same modulatory signaling patterns. This yields, however, opposite end results where a positive correlation with an overall fat metabolism indicates that BMAT, in fact, actively induces bone mass loss and inferior quality.

Both, over and malnutrition represent systemic changes in energy metabolism, affecting bone fat volume and bone mass. Despite the fact that adipose (overweight) individuals present with a higher body weight, the more often than not demonstrate a lower BMD. In obese older men and women, an enhanced percentage of body fat and low amount of lean (muscle) mass predicts a lower BMD and thus an enhanced frailty risk [14]. The enhanced fracture risk incurred by postmenopausal women and older men [15] is mainly due to enhanced circulatory levels of adipose tissue derived in adiponectin and proinflammatory cytokines, with a concomitant

lower secretion of leptin and IGF-1. Furthermore, one will often detect lowered blood levels of 25-hydroxyvitamin D and higher serum parathyroid hormone levels in these "patients" [15].
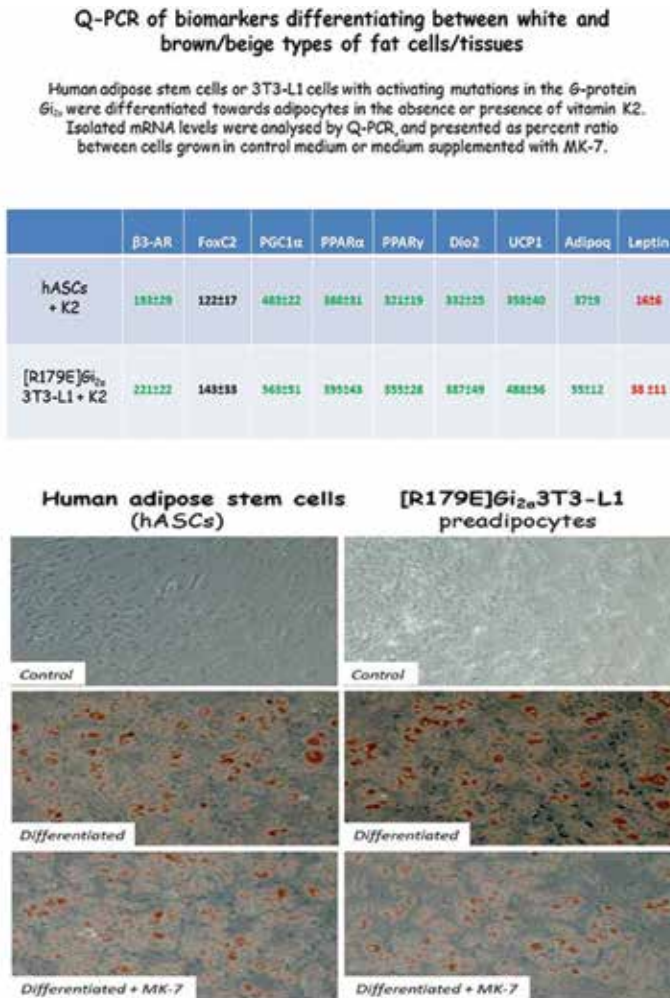


**Q-PCR of biomarkers differentiating between white and brown/beige types of fat cells/tissues**

Human adipose stem cells or 3T3-L1 cells with activating mutations in the G-protein Gi$_{2a}$ were differentiated towards adipocytes in the absence or presence of vitamin K2. Isolated mRNA levels were analysed by Q-PCR, and presented as percent ratio between cells grown in control medium or medium supplemented with MK-7.

| | β3-AR | FoxC2 | PGC1α | PPARα | PPARγ | Dio2 | UCP1 | Adipoq | Leptin |
|---|---|---|---|---|---|---|---|---|---|
| hASCs + K2 | 151±29 | 122±17 | 482±22 | 366±31 | 321±19 | 332±25 | 358±40 | 87±8 | 16±6 |
| [R179E]Gi$_{2a}$ 3T3-L1 + K2 | 221±22 | 143±18 | 568±51 | 395±43 | 555±28 | 887±49 | 488±56 | 55±12 | 38±11 |

**Human adipose stem cells (hASCs)** | **[R179E]Gi$_{2a}$3T3-L1 preadipocytes**

Control | Control

Differentiated | Differentiated

Differentiated + MK-7 | Differentiated + MK-7

**Figure 1.** Human adipose stem cells and mutated 3T3-L1 preadipocytes were differentiated towards mature adipocytes. *Top panel*: Control cells are not manipulated with differentiating medium show lack of ability to store lipids (no Alizarin Red accumulation). Cells are differentiated in the presence of insulin, as well as IBMX (an inhibitor of phosphodiesterase) accumulate lipids, while cells are also exposed to vitamin K2 (MK-7) and lose their ability to produce and store triglycerides. *Bottom panel*: Percentage modulation relative to the "control" stage seen with exposure of human adipocytes and mutated 3T3-L1 cells to differentiating medium versus exposure to vitamin K2 (MK-7). Cells treated with vitamin K2 exhibit significant increments in genes characterizing beige adipocytes, where the alterations in PGC1α, PPARα, PPARγ, Dio2, and UCP1 expression were more prominent.
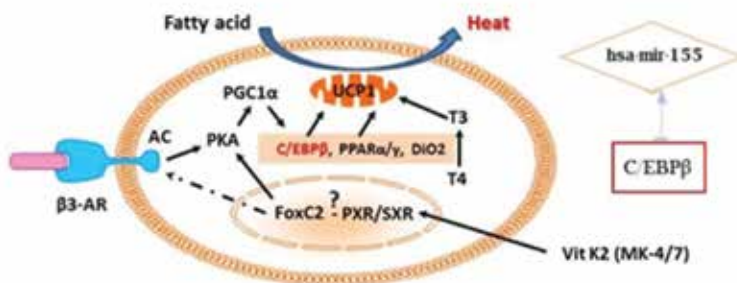
**Figure 2.** Putative model system featuring the mechanism behind the impact of vitamin K2 (MK-7). Based on previously published consensus material, our new findings of the effect of vitamin K2 (MK-7) shows that K2, via binding to the transcription factor PXR/SXR affects several signaling molecules and/or pathways, via the β3-adrenergic system, impinging on signaling molecules like PKA, PGC1α, C/EBPβ, and Dio2, which eventually affects the activity of the uncoupling protein UCP1, which produces heat (and not ATP) from fatty acids. Not mentioned in the text is the reciprocal regulatory loop consisting of the microRNA species hsa-mir-155 and the transcription factor C/EBPβ, which can be manipulated to reinforce the "beige" phenotype of the adipocyte after differentiation from the stem cells or preadipocytes.

## 2. Vitamin K2 and its mechanism of action – beyond xenobiotic metabolism

The steroid and xenobiotic receptor (SXR), which is synonymous with the pregnane X receptor = PXR, is characterized as a nuclear hormone receptor that is stimulated by a plethora of hormones, dietary steroids, pharmaceutically active agents, as well as xenobiotic compounds. SXR exhibits a binding domain, which diverges across mammalian species. SXR is construed as a xenobiotic sensor, facilitating xenobiotic clearance in the liver and intestine. However, newly published experiments unravel novel roles for SXR in modulating phenomena like inflammation, bone turnover, metabolism of vitamin D, lipid, and energy homeostasis, as well as cancer. The characterization of SXR as conveyer of hormone-like signals has now been recognized as a key instrument for the study of novel mechanisms, through which diet may ultimately affect health and disease. The discovery and pharmacological development of new PXR modulators, like vitamin K2, might represent an interesting and innovative therapeutic approach to combat various ailments and diseases.

Without elaborating on the details of how different natural products modulate the activity SXR to affect gene expression, it should be noted that St. John's wort (hypericum perforatum), vitamin E (tocopherols and tocotrienols), as well as sulforaphane and *Coleus forskohlii*, the latter producing forskolin which is able to stimulate adenylate cyclase activity, suffice to say that K2 emerges as an interesting "player" on the scene featuring major metabolic pathways accounting for a plethora of actions to be reckoned with [16]. One good example is the effect of SXR activation on the metabolism of cholesterol and lipid turnover, a second one is its impact on the Fox transcription factors (i.e., FoxO1 and FoxA2), and their influence on energy homeostasis [16]. FoxO1 and FoxA2 are both members of the "forkhead" family tree of transcription factors, serving critical roles in both lipid metabolism, as well as gluconeogenesis in the liver [17]. FoxO1 stimulates hepatic gluconeogenesis during fasting through the activation of gluconeogenic genes, such as PEPCK1 (phosphoenolpyruvate carboxykinase 1), G6P (glucose-6-phosphatase), as well as and insulin-like growth factor-binding protein 1.

FoxA2, on the other hand serves as a key switch, representing one of the regulatory factors of the breakdown of hepatic fatty-acids during calorie-restriction (i.e., fasting). Via mammalian cell-based two-hybrid screening, it was feasible to identify FoxO1 as a coactivator of both CAR (constitutive androstane receptor)-mediated, as well as SXR-stimulated transcription [18]. FoxO1 may directly associate with CAR and SXR as a hormone-ligand-receptor complex and stimulate their transcriptional ability. And, both CAR and SXR function as corepressors of FoxO1, suppressing the FoxO1-mediated transcription by counteracting its association with its response elements within the susceptible genes. As well as obliterating the FoxO1 activity, drug-stimulated SXR and CAR species were also shown to downregulate HNF4$\alpha$ transcriptional power via suppression of PGC1$\alpha$, thus suppressing the transcription of both PEPCK1 and G6P [19]. This indicates that metabolic turnover of both drugs and glucose, the two major functions of the liver which are regulated independently, happens to be reciprocally coregulated via communication between xenobiotic sensors on one hand, and transcription factors in the liver, on the other. When blood sugar concentrations are rendered low due to fasting or subsequent to periods of exercise, the liver funnels energy rich molecules to extra-hepatic tissues and peripheral organs via either β-oxidation or the production of ketone bodies [20]. FoxA2 is stimulating both ketogenesis and β-oxidation via enhancement of the transcriptional activity of a plethora of genes, such as mitochondrial 3-hydroxy-3 methylglutarate-CoA synthase 2 (HMGCS2) and carnitine palmitoyltransferase 1A (CPT1A) in energy-depleted conditions (fasting), or subsequent to periods where extensive exercise prevails [21].

FoxA2 is phosphorylated and thus inactivated by the Akt pathway, and serves to decrease lipid turnover in response to insulin. Treatment with other drugs such as barbiturates has been shown to suppress lipid metabolism in an insulin-independent fashion [22]. Furthermore, it was reported that SXR may crosstalk with FoxA2 in order to induce the repression of lipid turnover in livers of fasting mice. By applying wild-type and SXR-/- animals, it was demonstrated that treatment with PCN (pregnenolone-16d-carbonitrile) diminished the steady-state mRNA levels of HMGCS2 and CPT1A in control animals, but not in SXR-deficient mice. When conducting biochemical and cell-based analyses, it was shown that SXR markedly downregulates the ability of FoxA2 to bring about activation of the HMGCS2 and CPT1A genes. SXR that serves as the ligand binding domain will associate directly with the DNA-binding area of

FoxA2, and the ensuing interaction will halt the "coupling" between FoxA2 and its response in DNA elements. The communication between SXR and FoxO1/FoxA2 signifies that SXR, apart from being a modulator of drug metabolism in the liver, is also serving as an important regulator in hepatic in glucose and energy homeostasis. Hence, since vitamin K2 binds to and enhances SXR-mediated transcription, it may serve as the natural "drug" to ingest, when one is aiming to treat insulin resistance and type II diabetes mellitus. The impact of vitamin K2 via SXR on the metabolic functions in general, is described in more detail in the forthcoming paragraphs ("SXR in glucose handling" and "SXR in lipid turnover") [22].

In **Figure 3**, we show our own experiments indicating that the adipocyte phenotype, characterized by the expression of the transcription factor PPARγ, is heavily dependent on the presence of FoxO1 and FoxA3, respectively, since siRNA against either of them completely obliterate the stimulatory effect of vitamin K2 obtained through its binding to SXR. However, the literature in general advocates a plethora of PIK3/Akt-stimulated transcription factors of the FoxA and FoxO families in the cascade of insulin/IGF-1 mediated signaling in general. However, the present data show, for the first time that vitamin K2 (i.e., the MK-7 variant) directly stimulates FoxO1 and FoxA3, short-cutting the insulin/IGF-1 activation cascade. The assertion to be drawn is simply: Vitamin K2 may directly fortify the action of insulin, thus ensuring a better glucose homeostasis, as well as protection from a detrimental turnover of lipids and protein structures of the body.
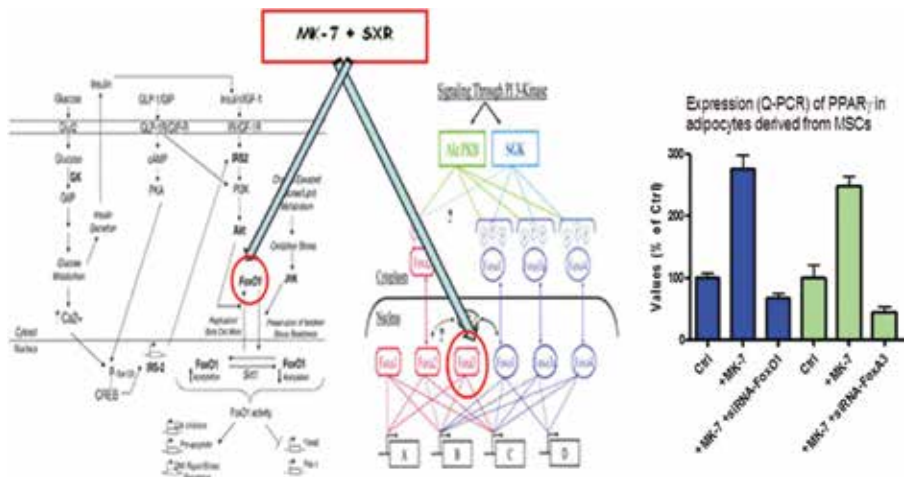


**Figure 3.** The impact of vitamin K2 (in the form of MK-7) on the signaling mechanism of insulin, via the PIK3/Akt/FoxO/FoxA cascade. *Left panel*: MK-7, bound to the transcription factor SXR activates FoxO1 and FoxA3, with ensuing stimulation of Sirt1. *Right panel*: Expression (mRNA analyses) of the transcription factor PPARγ in adipocytes generated from stem cells, either stimulated by vitamin K2 (MK-7) or inhibited in the presence of siRNA against FoxO1 or FoxA3. Reference to the figures: Left – DN Gross et al. (Oncogene, 2320–2336, 2008); Michael P. Czech (PNAS, 11198–11200, September 30, 2003).

### 3. SXR cross talks with biological signals in biological responses: implications in health and disease

#### 3.1. The molecular mechanisms of SXR-mediated gene repression

Currently, SXR has been described as a repressor of gluconeogenic gene expression, some of which are glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase 1 (PEPCK1), thus implicating vitamin K2 in metabolic (energy-related) reactions taking place in the liver [23–25]. The SXR-vitamin K2 complex may therefore interfere directly with transcription factors and thus be rendered responsive to both insulin and glucagon. Consequently, one would observe a release of transcription factors with their coactivators from target genes, which would lead to a general subactivation of gene transcription.

Furthermore, it has been asserted that SXR represses the CYP-genes through interference with the vitamin D receptor, VDR [26]. Hence, in tissues short of vitamin D3, liganded SXR would associate directly to vitamin D response elements (VDREs) and modulate transcription. Therefore, it may be asserted that vitamin K2 and vitamin D, as well as vitamin A (via VDR and RXA, respectively), and many other transcription factor like molecules (e.g., PPARs, FXR, LXR$\alpha$, LRH-1 = NR5A2, RXR), when associated with their ligands, may act synergistically on gene transcription in general [16, 27–29]. Hence, it is not straightforward to predict the net results of a certain combination of liganded transcription factors on biological processes. However, there are several excellent reports on the impact of vitamin K2, in association with the nuclear factor SXR, on cellular metabolism.

#### 3.2. Involvement of SXR in metabolic functions

Xenobiotics are able to enhance SXR-mediated expression of xenobiotic-metabolizing enzymes in both the liver and intestines. Even though such a modulation normally serves to detoxify the xenobiotics in question, these "alien molecules" enhance the production of intermediates, which confer harmful attack of tissues on the body [30, 31]. Additionally, SXR affects the balance of endobiotics (e.g., steroid hormones, cholesterols, and bile acids) aided by the same biochemical pathways. Hence, an SXR activation will consequently stimulate a plethora of physiological responses, i.e., in the liver, which plays an important role in the processing of, among many substances, glucose and lipids. Disruption of their metabolic fate may result in diseases, of which type II diabetes (T2DM) and obesity are most frequently encountered. Newly published studies of SXR-KO and SXR-humanized animals clearly shed light on the metabolic functions of SXR in man.

#### 3.3. SXR and its role in energy metabolism of the liver

The liver provides energy sources to the rest of the body, i.e., carbohydrates and lipids are catabolized in order to fuel both central and peripheral tissues and organs. The effect of SXR on hepatic energy turnover was discovered with the aid of SXR-KO mice [25, 32, 33].

### 3.4. SXR in glucose handling

The pancreatic hormones (insulin and glucagon) reciprocally regulate the blood glucose level via transcriptional processes, where rate-limiting enzymes like G6Pase and PEPCK1 in the glucose metabolism play a decisive role [34–36]. The glucose-6-phosphatase dephosphorylates glucose-6-phospate (G6P), constituting the endpoint of both the glucose-forming and glucose-utilizing reactions, while PEPCK1 converts oxaloacetate to phosphoenolpyruvate (PEP) in the gluconeogenic reaction. These enzymes are instrumental in controlling blood glucose levels. During fasting and/or prolonged exercise, glucagon dominates glucose metabolism by activating the cAMP/PKA signaling pathway [35, 37, 38]. When phosphorylated by PKA, the cAMP-response element-binding protein (CREB) stimulates both the G6Pase and PEPCK1.

However, insulin acts in an opposite manner, and in response to high blood glucose levels, insulin is secreted and activates the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway [39]. Thereafter, Akt phosphorylates and inactivates the transcription factor forkhead box O1 (FoxO1). FoxO1, being a key regulator of glucose turnover, subsequently stimulates the insulin response sequence (IRS)-bearing genes G6Pase and PEPCK1 [40]. When phosphorylation by Akt, FoxO1 cannot longer translocate to the nucleus, it is rapidly acetylated and therefore loses its activity [41, 42].

But, when using SXR-KO and SXR-humanized animals, it has been demonstrated that SXR is an important regulator of xenobiotic-dependent glucose turnover in the liver. Treatment of mice with potent SXR activators consistently leads to lowered blood glucose levels in laboratory animals [32]. Furthermore, it was shown that there existed so-called "cross-talk" between SXR and FoxO1 as a molecular mechanism underlying the downregulation of glucose metabolism [18]. It was reported by Kodama and Negishi that liganded SXR directly interacts with phosphorylated CREB in primary hepatocytes [25], and that SXR disturbs the binding of CREB to CRE with an ensuing repression of the CREB-mediated transcription of G6Pase and PEPCK1 genes. Looking at hitherto available information, it can be asserted that SXR, by "targeting" a plethora of factors modulated by insulin and glucagon, leads to an activation of many genes being functional in the intrinsic regulatory machinery maintaining serum glucose levels within "healthy" limits.

### 3.5. SXR in lipid turnover

The liver provides lipid-derived energy-rich compounds to different parts of the body. Hepatic lipid metabolism is controlled by the net influence of the "reciprocal" hormones insulin and glucagon, as well as by nutritional conditions. Some 10 years ago, Kodama and Negishi, along with Nakamura [25, 32], published that treatment with PCN (an activator of SXR) decreased the mRNA levels of carnitine palmitoyltransferase 1a (CPT1a) and 3-hydroxy-3-methylglutarate-CoA synthase 2 (Hmgcs2) in livers of starved wild-type mice, but not in SXR-KO mice [32]. CPT1a is instrumental in the overall mitochondrial β-oxidation

by funneling long-chain fatty acids into mitochondria [43], and the mitochondrial enzyme HMGCS2 facilitates the initial reaction of ketogenesis [44].

Additionally, the stimulation of SXR by PCN has been demonstrated to enhance the mRNA steady state level of stearoyl-CoA desaturase 1 (Scd1) in hepatic tissue of starved wild-type experimental animals. SCD1, which serves as a key enzyme in hepatic lipogenesis, facilitates the rate-limiting step in the synthesis of unsaturated fatty acids [45]. The plasma concentrations of 3-OH-butylate were decreased, while the hepatic level of triglyceride (TG) was increased by the PCN treatment in wild-type mice during assay conditions. However, neither TG nor cholesterol levels in the blood were altered in those animals, despite the fact that there was a significant rise in TG accumulated in their liver. Hence, as a means of survival during fasting, SXR is thought to slow down hepatic lipid turnover by repressing β-oxidation and ketogenesis, while stimulating the transcription of lipogenic enzymes, in much the same way as induced by insulin.

The Akt-regulated forkhead transcription factor FoxA2 which serves as a facilitator of insulin-dependent modulation of β-oxidation and ketogenesis, enhances expression of both the CPT1a and HMGCS2 gene, respectively [21, 46]. It is well known that Insulin activates the PI3K/Akt signaling pathway to phosphorylate FoxA2, in order to translocate it from nucleus to cytosol, thereby downregulating both the genes. And, it has been asserted that a direct interaction between SXR and FoxA2 serves as the mechanism, by which SXR represses the transcription of CPT1a and HMGCS2 in the liver [32].

A plethora of transcription factors and coregulators have been asserted to serve as modulators of hepatic lipid metabolism, e.g., the peroxisome proliferator-activated receptors (PPARs), the liver X receptor $\alpha$ (LXR$\alpha$), as well as the sterol regulatory element-binding proteins (SREBPs) [47]. The expression of SREBP1c, which is construed as the dominant regulator of hepatic lipogenesis, is under the control of LXR$\alpha$, and mediates the insulin- and fatty acids-dependent responses of lipogenic genes such as fatty acid synthase (FAS), acetyl-CoA carboxylase 1 (ACC1), stearoyl-CoA-desaturase-1 (SCD1), and fatty acid elongase (FAE). SXR is believed to upregulate lipogenesis in the liver, independently of SREBP1c action, and it is not deemed to be associated with the steady state expression levels of both the Fas and Acc1 genes. Among the cluster of lipogenic genes, Cd36 (cluster of differentiation 36) is deemed to be serve as a direct target of SXR in the liver. And, upon stimulation by ligands, the receptor is believed to become recruited to a DR3-type SXR response element within its promoter region of the liver of experimental animals [48]. Furthermore, SXR has been asserted to serve as a link, facilitating the upregulation of the Ppar$\gamma$-gene, which functions as a strong regulator of lipid-synthesizing enzymes [48]. Such a cross-talk involving nuclear receptors should confer a significant impact on the body's lipid homeostasis. Our data are in line with the published literature, however, it should be asserted that SXR probably affects a larger spectrum of FoxO and FoxA species than those presented in this review. In this way, one might speculate that SXR is able to recruit a "moving" representation of these transcription factors simultaneously, and that the net effect on various cell phenotypes depends on: (1) the distribution of FoxOs and FoxAs at any time within the cell or tissue, as well as (2) the epigenetic machinery or "make up" at any time within the same cells or tissues.

## 4. The effect of vitamin K2 on other genes related to metabolic processes in the cell

In 2009, Slatter [49] and coworkers published a paper, featuring oligonucleotide microarrays with the intention to reveal the heterogeneity of drug metabolism associated gene expression in liver tissue from healthy humans. Their intention was to define clusters of so-called "absorption, distribution, metabolism, and excretion" = ADME genes to define subgroups of coregulated genes. When analyzing the gene sets, they discovered distinct patterns of "parallel" gene expressions featuring gene "clusters", which proved to be modulated by the nuclear receptor SXR. So called "fold range metrics and frequency distributions" were applied in order to reveal the variability of solitary PKDM genes. The most variable gene entities chiefly correlated to: (1) drug metabolism, (2) intermediary metabolism, (3) inflammation, and (4) cell cycle control. Unique expression patterns of these genes allowed for a further correlation with a parallel expression of a plethora of other genes. Of major interest was the identification of SXR responsive genes.

A comprehensive list of these genes can be found in the article, however, quite a few of which are related to metabolic processes in the cell. The genes are the following (in alphabetical order): CLOCK, DUSP7, GCDH, IGFBP2, MAP2K2, NUCB2, OGT, PFKB1, PTPN11, and SLC16A2. By "looking up" current descriptions of the genes in "Gene-Cards", the following features of these SXR-sensitive genes were obtained (of which parts of their description is cited as presented):

| Name of gene | Description of cellular function(s) http://www.genecards.org/cgi-bin/carddisp.pl?gene=NR1I2 |
|---|---|
| CLOCK | Clock circadian regulator (The protein encodes a transcription factor, and serves as DNA-binding histone acetyl transferase). *Interpretation:* Polymorphisms in this gene may be associated with obesity and metabolic syndrome. CLOCK normally regulates gene products (proteins) in an optimal fashion, adapted to diurnal demands on the body related to food ingestion, physical activity and recreation/sleep. |
| DUSP7 | Dual-specific phosphatase (DUSPs) constitutes a large subgroup of cysteine-base protein-tyrosine phosphatases characterized by their ability to dephosphorylate both tyrosine and serine/threonine residues. *Interpretation:* DUSP7 may function as a modulator of cellular exposure to insulin and growth factors, ensuring energy homeostasis within optimal limits. |
| GCDH | The protein encoded by this gene belongs to the acyl-CoA dehydrogenase family. It catalyzes the oxidative carboxylation of glutaryl-CoA to crotonyl-CoA and $CO_2$ in the degradative pathway of L-lysine, L-hydroxylysine, and L-tryptophan metabolism. *Interpretation:* GCDH is involved in the stability of mitochondria, and hence energy metabolism in general. |
| IGFBP2 | Insulin-like growth factor binding protein, type 2. This protein inhibits IGF-mediate growth. *Interpretation:* A reduction in IGFBP2 may be responsible for organ hyperplasia and the development of neoplasia (cancer). |
| MAP2K2 | This MAP-kinase catalyzes the concomitant phosphorylation of a threonine and a tyrosine residue in a Thr-Glu-Tyr sequence located in MAP-kinases. It activates the ERK1 and ERK2 MAP-kinases (by similarity). *Interpretation:* This kinase may block tumorigenesis and normalize energy metabolism via MEK1/2 and the FoxO- and FoxA-family of transcription factors. |
| NUCB2 | Anorexigenic peptide; seems to play an important role in hypothalamic pathways regulating food intake and energy homeostasis, acting in a leptin-dependent manner. *Interpretation:* Appetite regulator fortifying the effect of leptin, but independent of the size of fat depots. |

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| OGT | Glycosylates a substantial and diverse amount of proteins, encompassing species like histone H2B, AKT1 and PFK (phosphofructokinase). It can modulate their cellular processes through cross-talk between processes like glycosylation and phosphorylation, or via proteolytic processing. Involved in insulin sensitivity in muscle cells and adipocytes by glycosylating components of insulin signaling, blocks phosphorylation of AKT1, stimulates IRS1 phosphorylation, as well as attenuating insulin signaling. *Interpretation:* Modulator of insulin and IGF-1 signaling/sensitivity to maintain a healthy muscle tissue fat mass and distribution. |
| PFKFB1 | Encodes a member of the family of the bifunctional 6-phophofructo-2 kinase: fructose-2, 6-bisphosphatase enzymes. These enzymes form homodimers, which catalyze the synthesis, as well as the degradation of fructose 2, 6-bishosphate, via independent catalytic domains. Fructose-2, 6-bisphosphate serves as the activator of the glycolytic pathway, and as the inhibitor of the gluconeogenetic pathway. *Interpretation:* Regulating fructose-2,6-bisphosphate levels through the activity of this enzyme is thought to regulate glucose homeostasis. Multiple alternatively spliced transcript variants have been found for this gene. |
| PTPN11 | PTP (protein tyrosine phosphatase) is a member a large family of phosphatases and plays a regulatory role in various cell signaling events that are important for a diversity of cell functions, such as mitogenic activation, metabolic control, transcription regulation, and cell migration. *Interpretation:* Because of the activating effects of PTPN11 on ERK (extracellular signal regulated kinase), a lack of PTPN11 activation may lead to adiposity, diabetes, and hyperleptinemia. |
| SLC16A2 | Very active and specific thyroid hormone transporter molecule. Stimulates cellular uptake of thyroxine (T4), triiodothyronine (T3), reverse triiodothyronine (rT3), and diiodothyronine. *Interpretation:* Lack of SLC16A2 activation may lead to a reduction in uptake and biological functions of T4 and T3, which is associated with adiposity, diabetes, and hyperleptinemia. |

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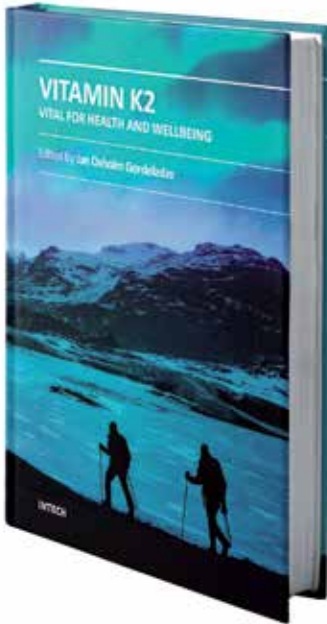
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