

The Role of Nuclear Receptor-FGF Pathways in Hormonal Regulation of Homeostasis

A. Introduction

A-1: Hormonal regulation of homeostasis

Health and lifespan in mammals are largely dependent upon their body's innate capacity to maintain relative constancy of its internal environment in a broad range of external conditions. This requires maintenance of a dynamic equilibrium between a large number of parameters such as body temperature, heartbeat, blood pressure, composition of body fluids, and energy metabolism. These parameters are held to a fixed value called the set point, which normally remains constant over time, but can be readjusted when the prevailing conditions are changed, e.g. the metabolic set point is lowered when the body is deprived of food. When such a parameter deviates from its set point, specific cell-surface and/or nuclear receptors located in various parts of the body are activated to produce a corrective feedback response to reestablish the original value. This is largely accomplished by readjusting the circulatory levels of relevant hormones through transcriptional activation or repression of genes involved in their biosynthesis, secretion, and degradation.

A-2: Nuclear Receptors

Nuclear receptors are a large family of structurally-related intracellular proteins that function as ligand-activated transcription factors. Ligands of these receptors include various lipophilic molecules, such as steroid and thyroid hormones, fat-soluble vitamins, cholesterol, and other lipids. All nuclear receptors share a common domain structure consisting of a centrally located DNA binding domain, a specific ligand binding domain within the C-terminal half of the receptor, a variable length hinge region between the DNA and ligand binding domains, as well as variable N-terminal and C-terminal domains. The DNA binding domain is responsible for recognizing and binding to short stretches of specific DNA sequences, termed hormone response elements, in the control region of target genes. Prior to ligand binding, nuclear receptors are typically associated

with other proteins that stabilize their latent conformation, protect them from proteolytic degradation, and fixate them either in the cytosol (type I receptors) or within the nucleus (type II receptors). Ligand binding to nuclear receptors induces a conformational change within the receptor molecule. In type I receptors, this triggers a number of downstream events that typically include dissociation of associated proteins, homo-dimerization, and recruitment of accessory proteins including chaperones that aid in guiding the receptor to target genes, and transcriptional coactivator or corepressor proteins, such as histone acetylases or deacetylases, respectively. Some of these events also occur subsequent to ligand binding to type II nuclear receptors. The end result of these events is formation of functional nuclear-receptor complexes that can facilitate or inhibit the transcription of specific genes. As such, nuclear receptors play a central role in the regulation of the body's development, metabolism and homeostasis.

A-3: Endocrine FGFs

The fibroblast growth factor (FGF) family contains 22 structurally-related proteins in mammals. Most of these proteins possess cell growth activity and function in a paracrine/autocrine fashion to promote tissue development, remodeling, and repair, as well as tumor growth and invasion. The rest constitute an atypical FGF subfamily, known as the FGF19 subfamily, whose members act in an endocrine fashion and are primarily involved in homeostatic regulation of metabolic parameters. Like other FGFs, members of this subfamily, comprised of FGF19 (the human ortholog of mouse FGF15), FGF21, and FGF23, signal through FGF receptors (FGFRs). However, unlike other FGFs, their interaction with FGFRs is critically dependent upon the presence of a functional Klotho protein as a co-receptor (Table 1). Klotho family proteins, including Klotho (also referred to as α Klotho) and β Klotho, exist both as membrane-bound and secreted proteins. These proteins function as key regulators of endocrine FGFs not only by promoting their binding to FGFRs, but also

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by determining the tissue-specific activity of these hormones. As discussed in the following sections, the expression of endocrine FGFs is regulated by specific nuclear receptors whose lipophilic ligands are regulated in turn by the specific action of these hormones in their target organs.

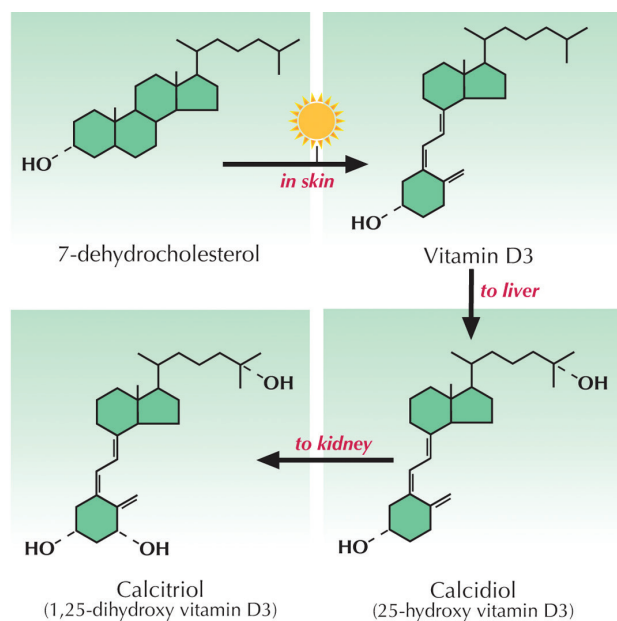
B: The regulatory role of VDR-FGF23 pathway in calcium/phosphate homeostasis.

Note: The phosphorus ions $\text{H}_2\text{PO}_4^{-1}$, HPO_4^{-2} , and PO_4^{-3} are collectively referred in this article as phosphate ions.

Regulation of calcium and phosphate homeostasis refers to the regulatory processes that maintain the levels of *free* calcium and phosphate ions in the extracellular fluid (ECF) within their normal physiological range. Since free calcium and phosphate ions are prone to form insoluble calcium phosphate deposits, failure to maintain the set point of either one of them may lead to adverse consequences including bone demineralization, ectopic calcification, infertility, and premature aging. The key hormones involved in calcium/phosphate homeostasis are parathyroid hormone (PTH), Calcitriol (also referred to as 1, 25-dihydroxyvitamin D3 or the active form of vitamin D), FGF23, and Calcitonin. PTH is an 84-residue polypeptide hormone produced and

stored in chief parathyroid cells which also express calcium-sensing receptors. These cells respond to a drop in calcium levels or hypocalcemia by secreting PTH, which acts to increase calcium levels by activating PTH receptors located in bone and kidney. In the bone, PTH action rapidly stimulates release of calcium ions from exchangeable pools of minerals surrounding the bone into the ECF. This PTH action is usually followed by a relatively slower osteoclast-mediated bone resorption. In the kidney, PTH promotes reabsorption of calcium ions from distal tubules and stimulates CYP27B1 gene expression. This gene encodes calcidiol-1 α -hydroxylase, the enzyme used in the conversion of calcidiol into Calcitriol. Renal production of Calcitriol is accomplished by 1 α -hydroxylation of 25-hydroxy vitamin D3 also known as calcidiol, an inactive form of vitamin D produced in the liver by hydroxylation of skin-derived vitamin D3 (Figure 1). Once secreted into the ECF, Calcitriol binds to transcalferrin, a liver-derived alpha-globulin that serves as a carrier for Calcitriol and other vitamin D metabolites and mediates their tissue uptake. In contrast to the relatively short half-life of circulating PTH (approximately 4 minutes), the half-life of transcalferrin-bound Calcitriol is 5-12 hours. Calcitriol acts to increase serum calcium by: (1) promoting intestinal absorption of dietary calcium, (2) enhancing the reabsorption of calcium filtered by the kidneys, and (3) mobilizing calcium from the bone to the ECF by stimulating bone resorption when absorption of dietary calcium is insufficient to restore or maintain normal calcium levels. Calcitriol action is regulated by a type II nuclear receptor called vitamin D receptor (VDR). Subsequent to Calcitriol binding, VDR forms a heterodimer with the retinoic acid X receptor, (RXR), and binds to Calcitriol response elements. The DNA-bound Calcitriol-VDR-RXR complex recruits transcriptional coactivator proteins to enhance the expression of genes involved in the negative feedback suppression of Calcitriol action. Among these are the FGF23 and Klotho genes, which are directly involved in regulating Calcitriol metabolism, as well as the GALNT3 gene that encodes a glycosyltransferase enzyme responsible for O-glycosylation of FGF23. This post-translational modification is essential for preventing rapid proteolytic degradation of circulating FGF23. Inactivating GALNT3 mutations cause familial hyperphosphatemia

Figure 1 - Biosynthesis of Calcitriol.



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Table 1: Endocrine FGFs: Function, Signaling Receptors, and Comments

Endocrine FGF	Function	Major Signaling Receptor	Comments
FGF19	Regulates bile acid metabolism	β Klotho -FGFR4	Produced in ileum under FXR regulation and acts in the liver.
FGF21	Regulates energy metabolism in ketotic state	β Klotho -FGFR1c	Produced in liver under PPAR α regulation and acts in white adipose tissue.
FGF23	Regulates Calcitriol metabolism and calcium/phosphate homeostasis	Klotho -FGFR1c	Produced in bone under VDR regulation and acts in kidney and parathyroid glands.

tumoral calcinosis, a rare autosomal recessive metabolic disorder associated with abnormally high serum phosphate levels and widespread ectopic calcification (1). A similar form of tumoral calcinosis is caused by different homozygous FGF23 missense mutations that affect conserved serine residues at O-glycosylation sites (2).

FGF23 is a bone-derived hormone that acts in kidney and parathyroid glands (four tiny glands around the thyroid gland) to regulate their response to hypocalcemia. FGF23 signaling, which is mediated by Klotho-FGFR1c, suppresses the activity of the renal sodium phosphate co-transporter NaPi-IIc, and regulates the key Calcitriol-metabolizing enzymes CYP27B1 and CYP24A1. As already mentioned, the former encodes the enzyme used in Calcitriol biosynthesis, whereas CYP24A1 encodes the enzyme that initiates degradation of Calcitriol through hydroxylation of its side chain. In the kidney, FGF23 exerts two seemingly opposing effects: (1) it inhibits NaPi-IIc-mediated phosphate reabsorption, resulting in increased excretion of phosphate ions into the urine, which shifts the ratio between calcium and phosphate ions in the ECF in favor of ionized calcium, and (2) it inhibits further release of Calcitriol from the kidney by inducing CYP24A1 gene expression, which leads to local degradation of Calcitriol and eventually to a shift in this ratio against ionized calcium. The short-term net result of these FGF23 effects is a sharp decline in ionized phosphate in the ECF. This prevents calcium ions obtained by the action of circulating PTH and Calcitriol from forming calcium phosphate deposits in the ECF. To reestablish calcium/phosphate homeostasis, FGF23 acts in parathyroid glands to prevent further release of PTH, the hormone that initiated the corrective response to hypocalcemia. There, it stimulates CYP27B1 gene expression, resulting in

local conversion of parathyroid-stored calcidiol into Calcitriol, which acts locally to suppress PTH expression and secretion. It should be noted that the quantity of Calcitriol that can be produced in the parathyroid is negligible compared to that produced in the kidney. The action of FGF23 in parathyroid glands constitutes a major negative feedback mechanism for controlling excessive action by PTH and PTH-induced Calcitriol in the bone, which may lead to severe bone demineralization and ectopic calcification. A rise in ionized calcium in the ECF above its set point induces thyroid secretion of Calcitonin, a hormone that promotes deposition of free calcium ions in bone.

C: The regulatory role of PPAR α -FGF21 pathway in energy metabolism.

Peroxisome proliferator-activated receptor alpha (PPAR α) is a nuclear receptor that regulates energy metabolism when the body is deprived of carbohydrates and fatty acids become its predominant energy source. Under such states referred to as ketotic states, liver-produced ketone bodies serve as an alternative fuel for the brain, which normally uses glucose as its sole energy source. Ketotic states may prevail in healthy subjects during periods of fasting, starvation, or consumption of low-carbohydrate diets. Ketone bodies, including β -hydroxybutyric acid, acetoacetic acid, and acetone, are water-soluble compounds produced from acetyl-CoA, mainly in hepatocytes when carbohydrates are scarce. Under ordinary conditions, acetyl-CoA produced in liver and other tissues by β -oxidation of fatty acids is further processed in the TCA cycle to produce ATP. In ketotic states, most of hepatic acetyl-CoA is converted into ketone bodies, which can be stored in hepatocytes or secreted to the blood. Usage of ketone bodies for ATP production is initiated by

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their reconversion into acetyl-CoA, a process that requires the action of β -ketoacyl-CoA transferase. This enzyme, which is expressed in many tissues throughout the body, is lacking in the liver to prevent futile cycles of hepatic synthesis and breakdown of ketone bodies.

The nuclear receptor PPAR α , whose natural ligands are free fatty acids, plays a pivotal role in the body's adaptation to starvation by lowering the metabolic set point through budgeted utilization of stored fat for energy. Ligand binding to PPAR α induces a conformational change which in turn triggers a cascade of events that includes: (1) receptor binding to DNA response elements in the promoter region of target genes, (2) formation of a heterodimer with the retinoic acid X receptor (RXR), and (3) recruitment of various transcriptional co-regulatory proteins. The resulting transcriptional complexes regulate the expression of numerous proteins including FGF21 and a battery of enzymes involved in energy metabolism. FGF21 is produced in the liver and acts in white adipose tissue to stimulate lipolysis and expression of GLUT-1, a glucose transporter that promotes insulin-independent glucose uptake (3). Signaling by this hormone is mediated by β Klotho-FGFR1c receptors, and involves binding of the C-terminal part of FGF21 to β Klotho and activation of FGFR1c by the N-terminal part of the protein (4). In addition to signaling through β Klotho-FGFR1c, FGF21 possesses high affinity binding to β Klotho-FGFR4, a receptor complex used for FGF19 signaling. However, unlike FGF19, FGF21 cannot activate β Klotho-FGFR4, which appears to act as a surrogate FGF21 receptor that regulates the release of this hormone into the blood. This receptor is expressed predominantly in the liver, the site of FGF21 production, but not in white adipose tissue, the site of FGF21 action. Another unusual FGF21-related phenomenon is the circadian variations in the expression of this hormone. It has been shown that a nighttime injection of a potent synthetic PPAR α agonist can effectively induce FGF21 expression, while a daytime injection cannot (5). Taken together, these unusual phenomena suggest that PPAR α acts in the liver in concert with β Klotho-FGFR4 to regulate energy expenditure during starvation by preventing FGF21-mediated release of fatty-acid fuel during daytime when mammals tend to be physically active, and allowing it to proceed only during

nighttime when mammals are usually asleep.

D: The regulatory role of FXR-FGF19 pathway in bile acid metabolism.

Bile acids are liver-produced biological detergents required for the generation of bile flow and excretion of lipid waste. In the gut, they facilitate absorption of dietary lipids and fat-soluble vitamins. Moreover, bile acid biosynthesis is the most significant pathway for the elimination of excess cholesterol from the body. The conversion of cholesterol to bile acids involves at least 14 enzymes, including Cyp7a1 which catalyzes the first and rate-limiting step in bile acid synthesis, i.e. hydroxylation of cholesterol at the 7 α -position. Subsequent to feeding-induced bile release into the duodenum, approximately 95% of the released bile acids are reabsorbed by intestinal epithelial cells (enterocytes). This process is mediated by the apical sodium-dependent bile acid transporter (ASBT), localized in the brush border membrane of enterocytes. Upon entry to enterocytes, bile acids bind reversibly to the intestinal bile acid binding protein (I-BABP), which transports them across the cells and releases them into the portal circulation to be carried back to the liver. Bile acid synthesis in hepatocytes, which is strongly stimulated by cholesterol feeding, is suppressed when their intracellular concentration reaches a level of about 1 μ M (discussed later). During prolonged fasting, bile acid synthesis and generation of bile flow are virtually diminished.

The detergent properties of bile acids render them intrinsically cytotoxic and therefore, their metabolism and transport must be tightly controlled. The regulation of bile acid homeostasis involves a large number of signaling molecules and a variety of nuclear and membrane receptors. The main target for controlling bile acid synthesis is the CYP7A1 gene, which encodes the rate-limiting enzyme, Cyp7a1, in the conversion of cholesterol to bile acids. CYP7A1 is regulated by the cholesterol sensor, liver X receptor alpha (LXR α), which acts in conjunction with liver receptor homolog-1 (LRH-1) to activate the CYP7A1 promoter. It should be noted that transcriptional activation of the CYP7A1 gene, which mediates the feed forward stimulation of Cyp7a1 and bile acid syntheses, is contingent

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upon the participation of both LXR α and LRH-1. The key regulator of the negative feedback suppression of Cyp7a1 and bile acid syntheses is the nuclear receptor, farnesoid X receptor (FXR). FXR, also known as the bile acid receptor, is highly expressed in the liver and gut, and to a lesser extent in kidney and adrenal gland. In the small intestine, bile acid activation of FXR induces the expression of the hormone FGF19, the bile acid transporter ASBT, and the orphan nuclear receptor SHP (small heterodimer partner). Intestinal-derived FGF19 acts in the liver to repress CYP7A1 promoter activity by inducing the expression of SHP which in turn, strongly interacts with LRH-1 and prevents it from acting as a competence factor for LXR α . As previously discussed, FGF19 signaling in the liver is mediated by the receptor complex, bKlotho-FGFR4. A recent study has shown that bKlotho-dependent FGF15/19 signaling in enterocytes activates the FXR-SHP-LRH-1 pathway to suppress the expression of the bile acid transporter ASBT (6). A FGF19-independent activation of this pathway is induced in hepatocytes by endogenously produced bile acids. This constitutes an intracellular feedback mechanism for the suppression of bile acid synthesis when their levels exceed the tolerable value of about 1 μ M. The FGF19-independent roles of FXR in the kidney and adrenal gland are beyond the scope of this article.

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