The endoplasmic reticulum (ER)-resident basic leucine zipper (bZIP) transcription factor c-AMP responsive element binding protein H (CREBH/CREB3L3) is exclusively expressed in the liver and intestine. Physiologically, CREBH is intrinsically linked to nutritional homeostasis via its regulation on fatty acid β-oxidation, lipid droplet process, very low-density lipoprotein metabolism, gluconeogenesis, and iron metabolism. Pathologically, CREBH enhances hepatic acute-phase response gene expression (e.g., C-reactive protein and serum amyloid P-component) and mediates nutrient-surplus induced metabolic inflammation. Hyperactivation of CREBH in metabolic inflammation further contributes to the development of hyperlipidemia, lipotoxicity, non-alcoholic fatty liver disease, and potentially non-alcoholic steatohepatitis. This review highlights recent findings that delineate the interactions between CREBH and peroxisome proliferator activated receptor α (PPARα), fibroblast growth factor 21 (FGF21), fat-specific protein 27 (FSP27), and lipoprotein metabolism with a focus on the molecular and biochemical mechanisms that underlie the development of metabolic inflammation, non-alcoholic fatty liver disease and inflammatory associated bone disease.

1. Introduction

1.1. The CREB Family of Transcription Factors

The cAMP responsive element binding protein (CREB) family of transcription factors (TFs) are defined by their specific affinity for the CRE-binding domain present in gene promoter regions. CREB TFs are typically activated by phosphorylation at serine residue 133 by either: a cAMP-dependent protein kinase (PKA); protein kinase C (PKC), p90 ribosomal s6 kinase (p90RSK), or the calcium flux sensitive calmodulin kinases.[1] Phosphorylation of CREB TFs allows interaction with CREB binding protein (CBP) or P300 to facilitate genetic regulation of their target genes. CBP and P300 assists transcription through acetylation of histones, enhancing accessibility of chromatin, and recruiting factors necessary for RNA polymerization.[2] The CREB family consists of transcription factors: CREB1 (CREB), CREB2 also referred to as activating transcription factor 4 (ATF4), CREB3, CREB3L1-4, and CREB5. The CREB family is fundamentally involved in various cellular metabolic processes, including lipid and carbohydrate metabolism and metabolic inflammation. Table 1 summarizes the major pathophysiological functions of the CREB family. It should be noted that the CREB3 family of TFs (CREB3 and CREB3L1-4) are highly conserved amongst metazoa with near identical DNA binding domains, suggesting they share overlapping functions and may only differ in their tissue distribution. Information provided in Table 1 further reveals that CREBH is the most investigated CREB family member. Although CREBH is exclusively expressed in the liver, small intestine and pyloric stomach, it also plays a significant role in metabolic systems and therefore will be the focus of this review.

1.2. Molecular Structure, Tissue Distribution, and Activation of CREBH

The CREB 3-like protein 3 (CREB3L3 in human /CREBH in mouse) is a basic leucine zipper (bZIP) TF of the CREB3 family. All CREB3 TFs share the bZIP domain and derive their name from their targeting CRE-domains and B-boxes; bZIP TFs bind DNA only as dimers, with dimer structure affecting TF-promoter cognizance, that is, whether the dimer is homo or hetero in nature affects the consensus sequence to which the bZIP-TF-dimer may recognize.[12] Such dimerization allows CREB3 TFs to exert finely tuned but far-reaching control over multiple overlapping metabolic pathways.
Table 1. Function of CREB transcription factors in prominent metabolic and inflammatory pathways.

| Protein | AMPK signaling | Glucose metabolism | Insulin signaling | Glucagon signaling | PI3K-Akt signaling | Insulin resistance | Lipid metabolism | Lipid signaling | NAFLD ER-stress signaling | TNF signaling | Bone development | Oestrogen signaling | Iron metabolism | Circadian Rhythm | Apoptosis inhibition | Apoptosis induction | MAPK signaling | Tissue distribution |
|---------|----------------|-------------------|------------------|-----------------|------------------|-------------------|------------------|----------------|-------------------------|--------------|------------------|-------------------|----------------|----------------|----------------|----------------|----------------|------------------|
| CREBH (mouse) | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| CREB3L3 (human) | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| CREB1 | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| ATF4 | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| CREB3 | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| CREB3L1 | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| CREB3L2 | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| CREB3L4 | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| CREB5 | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |

The above-illustrated functions of CREB5 are based on orthologous functions consistent amongst all CREB proteins as mapped in pathways found at KEGG:K09047.

Of the 55+ characterized human bZIP TFs, the CREB3 family exists within the larger OASIS family as determined by sequence homology within the coiled-coil region. Additionally, the CREB3 family is particularly closely related to both the activating transcription factor 6 (ATF6) and sterol-responsive element-binding proteins (SREBPs), but are differentiable by presence of ≈30 conserved residues adjacent to the bZIP N-terminal termed the adjacent to bZIP (ATB) domain. This unique domain is essential to the function of CREB3 and is conserved within a region not typically required for DNA binding, suggesting intentional conservation of a specific aspect of gene targeting or transcriptional activation.

CREBH was initially identified as liver specific, being particularly enriched in hepatocytes, it was later identified to express in the intestines, and to a lesser extent, in the pyloric stomach. Under homeostatic conditions, inactive CREBH rests in the endoplasmic reticulum (ER) and is maintained there by a transmembrane domain located in the N-terminal of the bZIP domain. Retention of CREBH in the ER is maintained through interaction between the ER and a cytosolic determinant termed the ER-retention motif (ERM); CREBH variants lacking the ERM can be efficiently transported to the Golgi apparatus and activated by cleavage independent of luminal determinants, which differs from other ER transmembrane transcription factors (i.e., ATF6).

Conditions that activate CREBH facilitate the TF migrates to the Golgi apparatus, where the CREBH N-terminal is cleaved by site-1 protease (S1P) (at the luminal site) and site-2 protease (S2P) (at the intramembrane site) via N-linked glycosylation. The cleaved N-terminal then migrates through the cytosol to the nucleus to mediate gene expression as a TF (Figure 1). Additionally, posttranslational modification by lysine acetylation at residue 294 has also been shown to be essential for transcriptional activation of CREBH. CREBH is acetylated during fasting and deacetylated in a fed state, both occurring in a time-dependent manner. Such acetylation and deacylation are performed by lysine acetyltransferase P300/CREB-binding protein-associated factor and histone deacetylase sirtuin-1 (SIRT1), respectively.

This review will highlight the essential roles of CREBH in lipid and lipoprotein metabolism, fatty acid β-oxidation, gluconeogenesis, metabolic inflammation and the associated metabolic syndrome. Metabolic pathways interacted with CREBH activities are summarized in Figure 2.

2. Metabolic Signaling Regulates CREBH Activation

Regulation of CREBH expression is particularly complex, occurring via a combination of circadian rhythm, prandial status, ER stress as a result of nutritional imbalance, and innate immune challenge (Figure 1). This is further complicated by tissue specificity of CREBH. In the liver, hepatocyte nuclear factor 4α (HNF4α) (in complex with PGC1α) is essential for hepatic expression of CREBH. However, gastrointestinal expression of CREBH is independent of HNF4α; instead, the gastrointestinal enriched HNF4γ is suggested to act as an HNF4γ substitute for intestinal CREBH expression.
2.1. Energy Switch between Fasted and Fed States Activates CREBH

The rapidly fluctuating nutritional demands of mammals are met by nutritional-state dependent signaling pathways that homeostatically shift metabolic processes between anabolic and catabolic states. The primary stimuli affecting these pathways is circulating glucose concentrations, which alter secretion of antagonistic peptide hormones insulin and glucagon. In a fed-state, glucose stimulates insulin secretion, promoting glycogenesis, and lipogenesis; contrastingly, starvation induces glucagon secretion, promoting lipolysis, glycolysis, and gluconeogenesis. Metabolic homeostasis in a fasting state is maintained by catabolism of endogenous triglyceride (TG) stored within cellular lipid droplets (LDs) of white adipose tissue (WAT). The catabolized TGs are freed into circulation as either free fatty acid (FA) or glycerol in an adipose triglyceride lipase (ATGL) and co-activator comparative gene identification-58 (CGI-58/ABHD5) dependent process.\textsuperscript{[25,26]} In this regulation, adipose tissue-derived FAs is essential for signaling expression of hepatic genes regulated by CREBH/PPAR\(\alpha\). Defective adipose tissue lipolysis in mice lacking ABHD5 or ATGL severely impairs PPAR\(\alpha\) and CREBH co-regulated gene expression in the liver due to reduced nuclear (active) CREBH.\textsuperscript{[27]} On the other hand, Crebh-null mice demonstrate severe reduction in hepatic G0s2 expression.\textsuperscript{[28]} In the fasting state, its expression is increased in the liver. Crebh-null mice display defective lipolysis within adipose tissue due to down-regulation of G0S2.\textsuperscript{[28]}

CREBH acts intracellularly downstream of glucagon to mediate expression of downstream signaling cascades to prevent ER stress, which, however, is repressed by insulin.\textsuperscript{[24]} Hepatic CREBH activity can also be regulated by specific dietary compounds, such as the organosulfur R-\(\alpha\)-lipoic acid (LA), which in turn induces transcription and translation of Insig-1 and Insig-2a to restrain the activation of sterol regulatory element binding protein (SREBP)-1c and the subsequent de novo lipogenesis.\textsuperscript{[29]}
2.2. Activation of CREBH by Inflammatory Cytokines Plays a Dual-Role in Metabolic Inflammation

CREBH was originally identified to be activated by ER stress. Pro-inflammatory cytokines, including tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-1β (IL-1β), can also induce CREBH activation. The regulation of CREBH on APR has been demonstrated by Zhang et al, who showed upregulation of Crebh mRNA induced by proinflammatory cytokines IL-6, IL-1β, and TNFα in a time dependent manner in the treated murine H2.35 hepatoma cells, which in turn stimulated expression of genes involved in acute inflammatory response. The bacterial endotoxin lipopolysaccharide (LPS) is also found to activate CREBH via the mediation of toll-like receptor (TLR)4-MyD88-TNF receptor-associated factor 6 (TRAF6) signaling. In this pathway, CREBH interacts with TRAF6, an E3 ubiquitin ligase that functions as a key mediator of TLR signaling which mediates K63-linked ubiquitination of CREBH to facilitate CREBH cleavage and activation. However, there is also evidence of CREBH negatively regulating immune responses in the liver by impeding expression of chemokines, and concomitantly the recruitment and infiltration of immune effector cells (e.g., neutrophils), via inhibition of P65, a nuclear factor (NF)-κB subunit. In a model of
ConA-induced hepatitis, Crebh-KO mice display elevated circulating TNFa, interferon-γ, and IL-1β and increased hepatic expression of P65 compared to wild type mice. Moreover, the expression of P65 in TNFa-stimulated primary hepatocytes is inversely proportional to CREBH level, and P65-mediated transactivation is inhibited by CREBH. This suggests that CREBH negatively regulates NF-κB signaling in immune responses via regulation of P65 expression. Whether this immune modulation also occurs in other CREBH expressing tissue (e.g., the gastrointestinal tract) remains undetermined.

3. CREBH Regulates Energy Metabolism

3.1. Interplay between CREBH and PPARα in Lipid Metabolism

The ligand inducible TF, PPARα, belongs to the peroxisome proliferator activated receptor family, of which there are three primary members: PPARα, PPARβ, and PPARγ. PPARα, and other PPAR TFs, only bind DNA as heterodimers, primarily heterodimerizing with retinoid receptors, such as retinoid-X-receptor (RXR). The PPARα-RXR heterodimer binds to the characteristic PPAR receptor element (PPRE) within genetic promoter regions of the target genes. Like CREBH, PPAR TFs are intrinsically linked to energy metabolic regulation and inflammatory signaling, playing an essential role in lipid transport, fatty acid oxidation, and ketogenesis (Figure 2).

CREBH and PPARα act as transient binary co-activators of one another, that is, a CREBH–PPARα complex may regulate expression of CREBH and PPARα, whilst also being capable of regulating expression of downstream metabolic signaling molecules. In rodent, expression of the rate limiting enzymes acyl-CoA oxidase 1 and EHHADH in fatty acid β-oxidation are controlled by PPARα. Endogenous ligands of PPARα, including saturated and unsaturated FAs, oxidized FA, oxidized phospholipid, and fatty Acyl-CoA species, activate PPARα and allow it to act as a key metabolic sensor of cellular FA concentration. This action is associated with the ability of CREBH to respond to FA-induced ER stress. PPARα positively induces CREBH expression via heterodimerisation with RXR; the PPARα-RXR complex binds to the aforementioned PPRE direct repeat element. Exogenous fatty acids, including palmitate, stearate, oleate, and linoleate, upregulated CREBH expression via both PPARα dependent and independent mechanisms. Oleate and palmitate induced CREBH in a PPARα independent manner.

3.2. CREBH Enhances Hepatic Fatty Acid β-Oxidation

Fibroblast growth factor 21 (FGF21) is an endocrine hormone primarily secreted from the liver. FGF21 is expressed during the fasting state by the synergistic transcriptional regulation of CREBH and PPARα. This enables pathways for energy saving that reduce growth and anabolic reactions whilst simultaneously enhancing sensitivity to insulin, increasing lipolysis, and promoting adipose tissue browning. The activity of FGF21 requires both the fibroblast growth factor receptor 1 and a coreceptor (β-Klotho).

CREBH is involved in the cellular recognition and response to FAs via the mediation of nuclear factors HNF4α and PPARα, which causes the nuclear transcription factors to illicit an appropriate trans-activation response. The CREBH/PPARα complex is essential for the expression of FGF21. Bhattacharya et al further demonstrated that CREBH mediated transcription of Fgf21 to be regulated by the E3 ubiquitin ligase (Hrd1) and its substrate recruiting cofactor, the single-spanning ER transmembrane protein suppressor/enhancer of Lin-12-like (Sel1L1). Protaosomal degradation of CREBH by Sel1L-Hrd1 ER-associated degradation (ERAD) under conditions of growth and feeding acts as a negative regulator on Fgf21 expression. This is evidenced by the significant increase of serum FGF21 (to ~10 ng mL⁻¹) in mouse models with Sel1L-Hrd1 ERAD deficient.

During fasting, the increased migration of TGs from WAT to hepatocytes enhances expression of hepatic fatty acid β-oxidation genes. CREBH support these processes by regulating various FA oxidizing and elongating enzymes (Figure 2): carnitine palmitoyltransferase 1a (CPT1a), a mitochondrial oxidizer of long-chain FAs responsible for facilitating the transportation of FAs across the mitochondrial membrane, is a known-target of CREBH-PPARα and is downregulated in Crebh-null mice. Furthermore, mRNA levels of the FA desaturases (Fads1/2) and FA elongases (Elov4/2/5) are decreased in Crebh-null mice.

3.3. The CREBH-FSP27 Axis in Lipid Droplet Metabolism

FSP27 was initially recognized as a cell-death inducing DFF45-like effector (CIDE) protein, however, it was later identified as necessary for proper LD formation and as a co-regulator of energy metabolism. LDs are subcellular organelles that store neutral lipids consisting of a hydrophobic TG-rich core surrounded by a phospholipid monolayer. Rodent FSP27 is highly expressed in both WAT and brown adipose tissue (BAT). CIDE is the homologue of FSP27 in humans. FSP27 possesses two mRNA isoforms, Fsp27α and Fsp27β, with the prior being dominant in WAT and the latter being dominant in hepatocytes and BAT. FSP27β is the more stable isoform with an additional 10 N-terminal amino acids. Gong et al. demonstrate FSP27α to be enriched at contact sites between LDs and to promote fusion of smaller LDs into larger LDs. Consistent with these observations, FSP27α deficiency induces accumulation of multiple small LDs in white adipocytes, contrasting with the large unilocular LD of standard white adipocytes.

Downregulation of PPARγ suppresses Fsp27 expression and promotes hepatic steatosis, suggesting PPARγ to control hepatic and WAT Fsp27 expression. In the liver, fasting-induced expression of Fsp27β is upregulated by the PKA-CREBH pathway. Human study further revealed that CIDEC is upregulated five folds in alcoholic steatohepatitis compared to healthy controls. Overexpression of Fsp27β induced TG accumulation, mitochondrial reactive oxygen species, and damage to hepatocytes in mice, which can be ablated by silencing of FSP27β, implicating that the CREBH-FSP27β axis may play a significant role in the development of hepatic steatosis. Taken together, the fasting-induced expression of Crebh and Fsp27β, and consequent accumulation of hepatic TGs upon forced expression of these genes suggests the CREBH-FSP27β axis may function to control lipid metabolism.
between adipose tissue and liver in the fasting state. During starvation, FAs are released in large quantities into circulation from WAT such that they can be transported to the liver for ketosis and maintenance of energy homeostasis, could they not be stored properly in LDs, they would cause lipotoxicity. Thus, aberrant activation of the CREBH-FSP27β pathway could contribute to the induction of hepatic steatosis.

3.4. CREBH in Very Low-Density Lipoprotein Metabolism

Chylomicrons and very low-density lipoprotein (VLDL) are TG-rich lipoproteins essentially for lipid transportation. In the fed state, TGs are primarily transported in chylomicrons secreted by small intestinal cells, whereas, in the fasting state, circulating TGs are transported in VLDL particles assembled in the liver microsomal compartment. Apolipoprotein B (APOB) is an amphipathic glycoprotein synthesized and secreted from the small intestine and hepatic ER. APOB synthesized in hepatocytes serves as a structure protein in the assembly of VLDL particles and a receptor ligand for the uptake of LDL lipoprotein remnant by the liver, whilst APOC2 and APOA5 play a role in facilitating TG hydrolysis from VLDL particles by stimulating lipoprotein lipase (LPL) activity in the periphery. Since higher risk of atherosclerosis is associated with increased plasma VLDL, the production of VLDL must be tightly regulated in order to meet metabolic needs. Maintaining physiological levels of CREBH is essential for hepatic VLDL assembly, secretion and lipoprotein remnant clearance. A study from our group demonstrated CREBH to be critical for VLDL-APOB biogenesis and the consequent secretion of hepatic lipids into periphery. CREBH binds to the CRE element in the APOB gene promoter, and subsequently enhances transcription of APOB mRNA whereas depletion of CREBH results in defective secretion of hepatic VLDL-APOB in response to inflammatory cytokine TNFα and high-fat diet, causing hepatic lipid accumulation, and steatosis. In the small intestine where chylomicrons are assembled, increased expression of CREBH in the intestine prevents high-cholesterol diet-induced hypercholesterolemia by reducing NPC1 like intracellular cholesterol transporter 1 (Npc1l1) expression, NPC1l1 being a cholesterol transporter specialized for transport of intestinal biliary cholesterol via a BMAL1-AKT-glycogen synthase 3β (GSK3β) cascade. Lysine acetylation is a process significant in CREBH transcriptional activity which displays typical circadian rhythmicity, peaking at 10:00 to 14:00, and troughing at 2:00. The dynamic expression pattern of Pck1 and G6pc mRNA is in accordance with the CREBH circadian cycle, further evidencing for the role of CREBH in regulating glucose metabolism. In line with this notion, in response to fasting, or during the daytime circadian phase, there is increased acetylation of CREBH at residue K294 which exerts its critical role in regulating glucose metabolism. Circadian and/or fasting mediated activation of CREBH further promote glycogenolysis by increasing expression of Pygl whilst gluconeogenesis is promoted under the same conditions by CREBH mediated upregulation of Pck1 and G6pc. Dysregulation of circadian rhythm is associated with development of insulin resistance.

3.5. CREBH Regulates Glucose Metabolism

During periods of fasting, the exceptionally high energy demands of mammals are subsidized by breakdown of endogenous glycogen via glycogenolysis in a liver glycogen phosphorylase (PYGL) dependent process. PYGL cleaves glucose monomers from the glycogen glycan, yielding glucose-1-phosphate. PYGL activity is regulated via allosteric activation by AMP and phosphorylation by PKA. PKA is a CAMP-dependent protein kinase, and CREB TFs are well established targets of PKA. CREBH gene expression is induced by PKA in a fasted state. Murine models in which hepatic adenoaviral CREBH is overexpressed showed elevated expression of gluconeogenesis genes and consequently, elevated plasma glucose concentration; whilst Crebh knockdown has been shown to reduce blood glucose concentrations. Barlow et al further observed CREB phosphorylation under oxidative stress to be PKA-dependent. During the fasting state, hepatic phosphoenolpyruvate carboxykinase 1 (Pck1) and glucose 6-phosphate (G6pc) are upregulated by CREBH. CREBH transcriptionally upregulates Pck1 by binding to the promoter CRE-element. Lee et al reveal that CREBH regulates hepatic gluconeogenesis by activating Pck1 or G6pc in a CREB regulated transcription coactivator 2 dependent manner. Emerging evidence further suggests that CREBH regulates glucose metabolism in a circadian manner via a BMAL1-AKT-glycogen synthase 3β (GSK3β) cascade.

Dietary restriction (DR), a reduction in caloric intake without malnutrition, has pleiotropic benefits that reduce cancer risk, inflammation, and potentially increase longevity. Comparable benefits of short-term DR can be induced by reduction of dietary protein intake [protein restriction (PR)]. Many of these benefits are attributed to alterations in lipid metabolism and reduction of VLDL secretion. A model has been proposed for CREBH-mediated PR-induced hypotriglyceridaemia: PR causes increased expression of Apoa5 and Fgf21, both of which are proposed to be controlled at a transcriptional level by CREBH/PPARs. Like all hepatic lipoproteins, APOA5 is embedded onto VLDL particles which serves as a co-regulator of LPL for clearance of TGs from the periphery by hydrolysis. This action is further enhanced by the upregulation of Fgf21. Such that, under PR-induced ER-stress, CREBH may facilitate clearance of VLDL from the circulation. These evidence emphasizes that CREBH may indirectly modulate metabolism of apolipoproteins to suppress inflammatory signaling. Aberrant activation of CREBH contributes to the pathological overproduction of VLDLs, leading to hyperlipidaemia and other complications.
and type-2 diabetes, thus, pathological activation of CREBH may contribute to the development of insulin resistance and type-2 diabetes.

4. **CREBH in Metabolic Inflammation Associated Diseases**

4.1. **CREBH Mediates Hepatic Acute Phase Response**

The APR is an innate immune response that is initiated in response to immunogenic antigens and host damage. CREBH is essential to the transcription of hepatic APR genes, including CRP and SAP, and acts synergistically with ATF6 to regulate the expression of these genes under ER-stress. CRP, an annular ring shaped pentameric protein, promotes phagocytosis by binding lysophosphatidylcholine expressed on surfaces of dead or dying cells. SAPs are pentraxins that bind amyloid motifs, including amyloid fibrils and immunoglobulin light chain, and can also bind to circulating pathogens. Both CRP and SAP expression is stimulated by proinflammatory cytokines IL-6, TNFa, and IL-1β. CREBH-null mice display trace level of CRP concentrations in both basal state and following exposure to pro-inflammatory cytokines, suggesting CREBH to be essential for APR mediated APR responses. On the other hand, the essentiality of CREBH for SAP production is debatable: Zhang and colleagues found reduced SAP concentrations in Crebh-null mice like that observed with CRP. In contrast, Lee et al found no reduction in SAP upon transient CREBH suppression. The inconsistent observations between these two studies may be due to the different animal models (e.g., genetic Crebh knockdown versus transient CREBH depletion) used in the studies. Regulation of Crp and Saps by CREBH has also been implicated in the anti-inflammatory effect of heme oxygenase-1-derived carbon monoxide in ER stress. CREBH positively regulates APR signaling whereas PPARα negatively modulates pro-inflammatory pathways and APR expression.

The orphan nuclear receptor oestrogen-related receptor γ (ERRγ) has been proposed to be a stimulator in ER-stress induced CREBH activation and the subsequent APR induction. Overexpression of Errγ both in vivo and in vitro enhances Crebh mRNA expression and protein activation. Conversely, knockdown of ERRγ impedes ER-stress activation of CREBH and up-regulation of Crebb mRNA expression, suggesting the interaction between CREBH and ERRγ in hepatic ER-stress response. Up-regulation of Crebh by ERRγ may occur via transcriptional regulation as a potential ERRE binding element (AGGTCA) is identified in Crebb gene promoter. Additional research is needed to further determine the biological significance of ERRγ on CREBH and the associated APR.

4.2. **CREBH in ER Stress and Metabolic Inflammation**

Under metabolic homeostasis, FAs must be esterified and compartmentalized in cellular lipid droplets, organelles most prominent in white adipose tissue. In times of increased metabolic demand, the FAs stored within LDs are released into circulation to meet cellular energy requirements. The maintenance of this system prevents tissues from being overwhelmed with otherwise harmful lipids. Constant consumption of excess dietary lipid can overwhelm adipocytes, causing inflammatory and metabolic pathologies. If insulin resistance occur under these conditions, the levels of basal lipolysis will increase, and suppression of fatty acid esterification pathways will reduce capability of cells to neutralize harmful lipids. A threshold exists beyond which lipid accumulation induces metabolic inflammation and lipotoxicity. The prior being a chronic low-grade metabolic inflammation associated with pathologies of metabolic syndrome, including type-2 diabetes and obesity and mediated by protein kinases PKC, C-Jun N-terminal kinase and PKR as well as CREBH and suppressor of cytokine signalling proteins; the latter being characterized by aberrant release of FAs causing WAT to develop an immunogenic phenotype characterized by increased expression of inflammatory cytokines and chemokines, such as TNFa and monocyte chemoattractant protein-1 (MCP-1), decreased anti-inflammatory adiponectin and adipokines and recruitment of macrophages and T-cells. CREBH has been demonstrated to be instrumental and indispensable in regulation of lipid homeostasis by regulating expression of genes in hepatic lipid metabolism, and consequently involved in metabolic inflammation.

Moreover, nutritional excess, particularly of free fatty acids, may cause metabolic inflammation by inducing ER stress. The ER is responsible for synthesis, folding, and modification of membrane and secretory proteins. When cytosolic conditions deviate from homeostatic norm, the ER must adapt. CREBH plays a dual-role in inflammation but also enhance the inflammatory response of this system prevents tissues from being overwhelmed with otherwise harmful lipids. Constant consumption of excess dietary lipid can overwhelm adipocytes, causing inflammatory and metabolic pathologies. If insulin resistance occur under these conditions, the levels of basal lipolysis will increase, and suppression of fatty acid esterification pathways will reduce capability of cells to neutralize harmful lipids. A threshold exists beyond which lipid accumulation induces metabolic inflammation and lipotoxicity. The prior being a chronic low-grade metabolic inflammation associated with pathologies of metabolic syndrome, including type-2 diabetes and obesity and mediated by protein kinases PKC, C-Jun N-terminal kinase and PKR as well as CREBH and suppressor of cytokine signalling proteins; the latter being characterized by aberrant release of FAs causing WAT to develop an immunogenic phenotype characterized by increased expression of inflammatory cytokines and chemokines, such as TNFa and monocyte chemoattractant protein-1 (MCP-1), decreased anti-inflammatory adiponectin and adipokines and recruitment of macrophages and T-cells. CREBH has been demonstrated to be instrumental and indispensable in regulation of lipid homeostasis by regulating expression of genes in hepatic lipid metabolism, and consequently involved in metabolic inflammation.

4.3. **CREBH in Non-Alcoholic Fatty Liver Disease**

Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease characterized by accumulation of lipids within hepatocytes. The condition is generally associated with hyperlipidaemia and metabolic inflammation, obesity and type-2 diabetes. NAFLD may progress to the inflammatory condition of non-alcoholic steatohepatitis (NASH) which severely impairs liver function due to chronic liver injury. Development and progression of NAFLD...
was initially believed to be a ‘two-hit’ process in which an initial insult of environmental factors, including a high-fat diet and sedentary lifestyle, cause desensitization of the liver to insulin and consequently disturb lipid metabolism.[27] This usually followed by a “second hit” which trigger chronic inflammation and fibrogenesis. However, due to the complex nature of the development of NAFLD, the two-hit hypothesis has been replaced by a “multiple hit” hypothesis in which a number of potential factors, including genetic and environmental, may act in parallel to induce insulin resistance and dyslipidemia prior to development of inflammation and fibrogenesis.[79–80]

The instrumentality of CREBH in developing NAFLD is associated with its essential role in hepatic lipid and lipoprotein metabolism. In humans, several genetic variants of CREBH have been identified to contribute to hypertriglyceridemia,[21,28,81] which in turn is associated with hepatic steatosis.[82] Several potential pathways exist for interaction of CREBH in NAFLD: Park et al have demonstrated accumulation of hepatic TG to induce CREBH expression in the liver which further stimulates expression of Fgf21 to act on adipose tissue and downregulate lipolysis, consequently restricting flow of non-esterified fatty acid (NEFA) to the liver and ameliorate hepatic steatosis.[83] Deacetylase SIRT1, a mediator of metabolic deacetylation, is known to be upregulated in obese patients with NAFLD. SIRT1 positively modulates PPARα activation and PPARγ, as described earlier, interacts with CREBH to regulate FA oxidation pathways.[49,86,87]

Whole body Ppara-null mice show impaired coping with prolonged fasting, causing hypoglycaemia, hypothermia, steatosis, and defective fatty acid β-oxidation.[88] Hepatic Ppara deletion hampers CREBH induced upregulation of Fgf21 expression in both fasting and fed state.[18] Conversely, non-functional mutations of Crebβ also impede regulatory activity of the ‘CREBH-FGF21’ axis on adipose tissue lipolysis and induce NAFLD due to compromised control of NEFA flux from adipose tissue to the liver.[85] CREBH further exerts control over fatty acid synthesis in the fasting state, or upon glucagon stimulation, by indirectly suppressing SREBP-1c activation via upregulation of insulin induced gene-2a (Insig-2a) and its sister protein Insig-1, two proteins that sequester SREBP-1c in the ER and prevent its migration to the Golgi apparatus to be activated. In Crebβ-null mice, inhibition of SREBP-1c by CREBH, results in a propensity to hepatic steatosis and progression to NAFLD.[24]

At the more advanced stage of NAFLD, such as non-alcoholic steatohepatitis (NASH), liver fibrosis, and cirrhosis, hepatic inflammation and severe liver injury are developed due to the increased secretion of pro-inflammatory cytokines by inflammatory responses and pro-fibrogenic components by hepatic stellate cells (HSCs).[89,90] CREBH deficiency has been shown to increase serum pro-inflammatory cytokines MCP-1 and TNFα, as well as apoptotic protein Bel-2 associated X protein (BAX). In Crebβ-null mice, expression of TGF-β1/2 and SMAD2/3 are increased in methionine and choline-deficient diet or high-fat diet induced liver fibrosis mouse models.[91] TGFβ is a prominent fibrogenic cytokine that stimulates apotosis of hepatocytes and fibrosis by promoting HSC proliferation.[92] SMAD2/3 mediates TGFβ signaling and promotes lipid accumulation via induction of lipogenic genes and suppression of fatty acid β-oxidation genes in hepatocytes. Silencing Smad2 was able to reverse the phenotypes induced by TGFβ signaling and depletion of SMAD3 protects mice from high-fat diet induced obesity, insulin resistance and steatosis.[93] Activation of the TGFβ and SMAD2/3 pathway was also observed in clinical patients with NASH.[94] Collectively, this evidence supports the protective nature of CREBH in a spectrum of chronic liver diseases from simple steatosis to NASH and further to liver fibrosis. However, a recent study also reported CREBH as a key positive regulator of TGFβ2 transcription in hepatitis C virus (HCV)-infected hepatocytes, leading to an increase in fibrogenic responses in adjacent HSCs.[95] This further emphasizes the complexity of CREBH singling in NAFLD.

4.4. CREBH in Inflammation-Associated Bone Disease

Bone morphogenic protein 2 (BMP2), an important stimulator of osteoblast differentiation, activates several UPR bZIP TFs, including ATF6, and OASIS. Bone formation by osteogenesis is inhibited by a variety of inflammatory cytokotnes, including TNFa, which has been implicated as a major mediator in the loss of bone in inflammatory diseases (e.g., osteoarthritis).[96,97] Jang et al identified CREBH as a key factor in TNFα-induced inhibition of osteogenesis.[98] The underlying mechanism is mediated by the IKK/IκBα/NF-κB signaling pathway in which the TNFa suppresses BMP-2-induced osteoblast differentiation via the CREBH/SMURF1/SMAD1 regulatory system. Interestingly, despite being closely related to both Oasi and Atf6 in the CREBH family, Crebβ mRNA expression in osteoblasts is not affected by BMP-2 but upregulated by TNFα: the exact opposite effect to that of BMP-2 and TNFα on Oasi and Atf6, suggesting the unique regulatory effect of CREBH in this regulation compared to other bZIP TFs.

Bone resorption is a molecular breakdown of bone for the purpose of repair and remodeling and is orchestrated by osteoclasts. Tumor necrosis factor (ligand) superfamily member (TNFSF11/RANKL), an indispensable stimulator of osteoclast differentiation,[99] induces ER-stress by producing long-lasting increases in mitochondrial reactive oxygen species production in osteoclast precursors, resulting in activation of CREBH[100] which in turn transcriptionally activates nuclear factor of activated T cells 1 (Nfatc1) to induce osteoclast. Wang et al further identified an insulin-like growth factor-binding protein 1 (IGFBP1) mediated bone resorption pathway stimulated by FGF21 in which FGF21 enhances hepatic IGFBP1 synthesis and secretion into plasma.[101] Osteoclast differentiation and bone resorption may be stimulated by IGFBP1 through potentiation of RANKL-induced Erk phosphorylation and NFATc1 activation, causing reduced bone mass.[101] Osteoporosis—structural deterioration of bone—is common in ageing and associated with inflammation; it is believed to originate from imbalances between bone formation and resorption.[102] The association between CREBH and TNFα and FGF21 in bone metabolism makes CREBH a interesting target for osteoporosis treatment: by reducing CREBH expression, one could potentially promote osteogenesis by preventing
TNFα-induced inhibition of osteoblast differentiation, and simultaneously inhibiting bone resorption by limiting FGF21 expression in the FGF21-IGFBP1 axis. Although being a significant regulator of FGF21, further investigation into the role of CREBH in the FGF21-IGFBP1 axis is needed.

5. Potential of CREBH as a Therapeutic Target in Metabolic Inflammation

There is a wealth of evidence for the role of CREBH in maintaining metabolic homeostasis and aberrant activation of CREBH leads to a propensity towards hepatic steatosis, NAFLD, metabolic inflammation, and associated bone disease. Given the dual-face and the tissue/organ specificity of CREBH in metabolic pathways, it is critical to tightly regulate expression and activity of CREBH signaling. For instance, recent reports on the role of CREBH in negatively modulating NF-κB activity by suppressing expression of NF-κB subunit p65, and consequently reducing expression of neutrophil attracting chemokines and neutrophil infiltration in severely inflamed livers.[31] This might suggest pharmaceutical upregulation of CREBH be beneficial for maintaining liver health in chronic liver disease by reducing recruitment of immune cells and consequently reducing inflammation in the liver. However, in the inflammatory associated bone disease, it appears that overexpression of CREBH may make an individual prone to elevated bone resorption as a result of increased differentiation of osteoclasts. Consequently, it is important that therapies aimed at targeting CREBH should bear in mind the tissue specificity and the fine-tuning of CREBH expression and activity.

6. Conclusion

CREBH is a major regulator of numerous essential metabolic pathways, serving to modulate ER-stress, maintain homeostasis of lipid, glucose, iron and bone metabolism, and to facilitate the APR. However, CREBH is a double-edged sword: activation of CREBH under physiological conditions is essential for metabolic homeostasis; In contrast, aberrant activation of CREBH under circumstances of chronic ER-stress or over-nutrition may trigger metabolic inflammation and the associated metabolic disease. For instance, pathological activation of the CREBH-VLDL-APOB and CREBH-FSP27 pathways by CREBH is associated with development of hyperlipidaemia, hepatic steatosis, and NAFLD. Contrastingly, at least in animal models of steatosis, upregulation, or forced upregulation of CREBH ameliorates disease symptoms. Whilst both genetic susceptibility and environmental factors play a role in the metabolic syndrome, environmental triggers seem the most prevalent means of developing CREBH-associated metabolic disease, particularly the nutrient-surplus as a result of excessive dietary caloric intake. Thus, most CREBH-associated diseases may be readily preventable, and it is paramount that we understand the dual-role of CREBH activity in both physiological metabolic homeostasis and disease developmental state.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

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