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FOXK1 and FOXK2 Orchestrate Transcriptional Networks Linking Insulin Signalling, Metabolic Reprogramming, and Mitochondrial Function

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Abstract

Insulin signalling coordinates cellular metabolism, growth, energy utilization, and adaptation to nutrient availability through interconnected kinase and transcriptional networks. Although FOXO transcription factors are well-established mediators of insulin-dependent transcriptional repression, increasing evidence identifies the forkhead transcription factors FOXK1 and FOXK2 as complementary nutrient-responsive regulators that integrate insulin signaling with metabolic and mitochondrial adaptation. FOXK1 and FOXK2 are regulated by insulin- and nutrient-sensitive signaling pathways involving AKT, mTORC1, and glycogen synthase kinase-3, resulting in changes in their subcellular localization, transcriptional activity, and downstream metabolic programs. Experimental studies indicate that FOXK1/FOXK2 regulate aerobic glycolysis, glucose utilization, lipid metabolism, mitochondrial respiration, and cellular proliferation. FOXK1 has particularly strong evidence for participation in hepatic insulin-responsive transcription, including direct occupancy of promoters and enhancers associated with metabolic and cellular programs. In parallel, FOXK1 contributes to mTORC1-dependent metabolic reprogramming through GSK3- and HIF1α-associated mechanisms. FOXK2 has emerging and context-dependent roles in mitochondrial bioenergetics and lipid metabolic remodeling, including regulation of fatty-acid oxidation and the mTOR/DRP1 axis in cancer models. Nutrient-sensitive post-translational mechanisms further expand FOXK function, as demonstrated by O-GlcNAcylation-dependent regulation of FOXK1, BAP1 recruitment, and E2F-associated proliferative transcription. Collectively, these findings support a model in which FOXK1 and FOXK2 act as partially overlapping but functionally specialized transcriptional integrators that coordinate glucose and lipid utilization with mitochondrial activity and cellular growth. Their biological effects are highly context dependent and vary according to tissue, nutrient status, signaling environment, transcriptional cofactors, and post-translational regulation. Despite substantial mechanistic progress, important questions remain regarding FOXK1/FOXK2 target specificity, tissue-specific functions, human genetic and clinical relevance, and the causal relationship between FOXK activity and metabolic disease. Integrated approaches combining genetic perturbation, transcriptomics, chromatin profiling, proteomics, phosphoproteomics, O-GlcNAc proteomics, metabolomics, metabolic-flux analysis, mitochondrial phenotyping, and human tissue validation will be required to define the FOXK regulatory network more precisely. FOXK1 and FOXK2 therefore represent promising biological nodes for understanding the intersection of insulin signaling, metabolic reprogramming, mitochondrial function, and disease progression, while their potential as therapeutic targets remains investigational.

Keywords: FOXK1, FOXK2, Insulin Signaling, AKT, mTORC1, GSK3, FOXO1, Metabolic Reprogramming, Aerobic Glycolysis, Lipid Metabolism, Mitochondrial Function, Fatty-Acid Oxidation, O-GlcNAcylation, Cancer, Metabolic Disease

1. Introduction

1.1. Insulin as an Integrator of Metabolism and Growth

Insulin is a major anabolic regulator that coordinates nutrient availability with glucose, lipid and protein metabolism, cellular growth, and survival. Its actions are mediated predominantly through the insulin receptor (IR), a receptor tyrosine kinase, with additional signalling through the closely related insulin-like growth factor-1 receptor (IGF1R). Ligand binding induces receptor autophosphorylation and phosphorylation of insulin receptor substrates (IRS1 and IRS2), which recruit downstream signalling complexes. Two major signalling branches are subsequently engaged: the phosphoinositide 3-kinase (PI3K)–AKT pathway, which mediates many metabolic effects of insulin, and the RAS–RAF–MEK–ERK pathway, which contributes prominently to growth and mitogenic responses [1,2]. PI3K activation generates phosphatidylinositol-3,4,5-trisphosphate, promoting recruitment and activation of AKT. Activated AKT regulates substrates controlling glucose transport, glycogen synthesis, lipid metabolism, protein synthesis, apoptosis, and transcription. AKT also promotes mechanistic target of rapamycin complex 1 (mTORC1) activity, thereby integrating growth-factor and nutrient signals with protein synthesis and anabolic metabolism. In parallel, AKT inhibits glycogen synthase kinase 3 (GSK3), an important regulator of glycogen metabolism and cellular signalling [1,2]. Thus, insulin signalling extends beyond acute metabolic control and establishes a signalling environment capable of producing sustained transcriptional and metabolic adaptations. Transcriptional regulation is particularly important for maintaining these longer-term responses. Insulin-responsive transcription factors convert transient kinase activation into changes in gene expression that persist beyond the initial receptor-proximal signal. The forkhead box (FOX) family, including FOXO and FOXK proteins, represents an important component of this regulatory network. Although FOXO1 has traditionally been regarded as a major transcriptional effector of insulin action, identification of FOXK1 and FOXK2 as insulin-responsive proteins has expanded the established insulin–forkhead signalling framework [3,4]. Defects in insulin signalling can also produce tissue-specific alterations in metabolic and growth pathways. This phenomenon, often described as selective or pathway-specific insulin resistance, is relevant to obesity, type 2 diabetes, metabolic dysfunction-associated liver disease, and cancer [2-5]. Defining transcription factors that integrate insulin signalling with metabolic and proliferative programmes is therefore important for understanding how hormonal signals are translated into tissue-specific cellular responses.

1.2. Forkhead Transcription Factors in Insulin Signalling

Forkhead box transcription factors constitute a large family of sequence-specific DNA-binding proteins characterised by a conserved forkhead DNA-binding domain. They regulate diverse biological processes, including differentiation, metabolism, proliferation, stress responses, apoptosis, and cellular homeostasis. Within insulin signalling, the FOXO subgroup has been particularly well characterised because of its direct regulation by AKT [3-6]. FOXK1 and FOXK2 represent a distinct branch of the FOX family. In addition to their forkhead DNA-binding domains, FOXK1 and FOXK2 contain forkhead-associated (FHA) domains, which function as phosphopeptide-recognition modules and provide a structural basis for interactions with phosphorylated regulatory proteins [7,8]. These features allow FOXK proteins to integrate DNA binding with protein–protein interactions and chromatin-associated regulatory complexes.

The identification of FOXK1 and FOXK2 as insulin-responsive factors provided an important extension of the classical insulin–FOXO model. Unlike FOXO1, whose nuclear activity is generally reduced following insulin-dependent AKT phosphorylation and nuclear exclusion, FOXK1 and FOXK2 undergo insulin-associated nuclear accumulation. Thus, insulin does not impose a uniform regulatory response across the FOX family, rather, individual FOX proteins are controlled according to their distinct signalling and structural properties [4].

This distinction has important metabolic implications. Experimental depletion of FOXK1 and FOXK2 in hepatic cells alters transcriptional programmes related to lipid metabolism, mitochondrial function, proliferation, and cellular survival. Combined depletion also reduces fatty-acid oxidation and mitochondrial respiratory capacity, indicating that FOXK proteins participate in the transcriptional regulation of cellular energy metabolism [4].

Evidence from genome-wide chromatin analysis has further established FOXK1 as a component of the hepatic insulin-responsive transcriptional network. ChIP-seq studies identified FOXK1 occupancy at more than 4000 proximal promoters and enhancers in hepatic cells, with insulin increasing FOXK1 association at a substantial proportion of these sites. FOXK1-bound genes include pathways involved in glucose and lipid metabolism, mitochondrial function, cell-cycle regulation, autophagy, and steroid biosynthesis. FOXK1 therefore appears to function as a broad transcriptional mediator of hepatic insulin responses rather than as a regulator of a single metabolic pathway [9].

1.3. FOXO1 as the Classical Insulin-Responsive Forkhead Factor

FOXO1 remains the best-established forkhead transcriptional mediator of insulin-dependent control of hepatic glucose metabolism. During fasting or reduced insulin signalling, FOXO1 accumulates in the nucleus and promotes transcription of genes involved in hepatic glucose production, including *PCK1* and *G6PC* [3,6].

Insulin stimulation activates the IR–IRS–PI3K–AKT pathway, resulting in phosphorylation of FOXO1 at regulatory sites. Phosphorylated FOXO1 associates with 14-3-3 proteins and undergoes nuclear exclusion, thereby reducing access to chromatin and suppressing FOXO1-dependent transcription. This AKT–FOXO1 mechanism provides a well-established molecular link between extracellular insulin concentrations and hepatic

gluconeogenic gene expression [3,6].

However, FOXO1 does not act in isolation. DNA-affinity purification and mass-spectrometry analyses identified FOXK1 among several insulin-responsive transcription factors associated with regulation of the *PCK1* and *G6PC* promoters. Insulin increased FOXK1 recruitment to the *PCK1* promoter, and FOXK1 depletion reduced hepatic glucose production and impaired the ability of insulin to suppress glucose production in primary hepatocytes [10].

Subsequent genomic analyses demonstrated that FOXK1 and FOXO1 share binding at a subset of hepatic genes but also occupy largely distinct genomic regions. FOXK1 binding was identified at *PCK1*, *G6PC*, and other genes involved in glucose metabolism, whereas overlapping FOXK1–FOXO1 occupancy was observed for selected genes involved in mitochondrial translation and other metabolic processes [9]. These findings support a model in which FOXK1 and FOXO1 can function in complementary or cooperative ways rather than as strictly opposing transcription factors.

Importantly, the role of FOXK1 in hepatic glucose regulation should not be interpreted as equivalent to that of FOXO1. FOXO1 has a well-established causal role in insulin-regulated gluconeogenesis, whereas FOXK1 appears to contribute to a broader insulin-responsive transcriptional network. The available evidence therefore supports functional interaction between the two factors while retaining distinct biological roles for each [9,10].

1.4. Emergence of FOXK1 and FOXK2 as Insulin-Responsive Transcription Factors

The identification of FOXK1 and FOXK2 as downstream targets of insulin signalling substantially expanded the insulin–forkhead paradigm. Using proteomic analysis of insulin receptor-associated proteins, Sakaguchi and colleagues identified FOXK1 as an insulin-responsive component of the receptor-associated signalling network. Insulin stimulation promoted FOXK1 redistribution from the cytoplasm towards the nucleus and chromatin. FOXK2 exhibited a related response, although its basal intracellular distribution differed from that of FOXK1 [4].

The direction of this response is reciprocal to that of FOXO1. Insulin promotes FOXO1 nuclear exclusion while favouring nuclear accumulation of FOXK1 and FOXK2. This arrangement provides a potential mechanism through which insulin simultaneously represses fasting-associated transcriptional programmes and activates distinct metabolic and growth-associated programmes [4,9].

Mechanistically, insulin-induced FOXK1 nuclear accumulation depends substantially on PI3K–AKT–mTOR signalling. Inhibition of PI3K or AKT attenuates insulin-induced FOXK1 nuclear accumulation, whereas mTOR inhibition interferes with this response. By contrast, GSK3 activity contributes to basal cytoplasmic retention of FOXK proteins, and GSK3 inhibition promotes their nuclear accumulation [4]. These findings place FOXK1/2 downstream of a signalling network in which AKT, mTOR, and GSK3 collectively regulate FOXK subcellular distribution.

Phosphoproteomic analyses identified insulin-responsive phosphorylation sites in FOXK1 and FOXK2, including sites compatible with GSK3-dependent regulation. These observations support a phosphorylation-dependent mechanism for controlling FOXK localisation, however, the precise contribution of individual phosphorylation sites and their relative dependence on GSK3 or other kinases requires further mechanistic definition [4]. This distinction is important because the available evidence establishes pathway dependence more strongly than it establishes a complete residue-specific molecular mechanism.

The functional consequences of FOXK perturbation extend beyond subcellular localisation. Depletion of FOXK1 and FOXK2 alters genes involved in lipid metabolism, mitochondrial function, oxidative phosphorylation, proliferation, and apoptosis. Combined FOXK1/2 depletion produces marked changes in fatty-acid oxidation and mitochondrial respiration, supporting a role for these transcription factors in metabolic adaptation [4].

Independent experimental work has further demonstrated that FOXK1 and FOXK2 regulate aerobic glycolysis. Increased FOXK1/2 activity promotes expression of glycolytic machinery and increases glucose utilisation and lactate production, whereas suppression of FOXK1/2 produces the opposite metabolic phenotype. These findings, obtained in cellular, animal, and primary human-cell models, indicate that FOXK1/2 can promote a glycolytic metabolic state under appropriate cellular conditions [11].

Collectively, these findings position FOXK1 and FOXK2 as transcriptional effectors that connect insulin-responsive signalling with broader metabolic and cellular programmes. Their functions encompass mitochondrial metabolism and fatty-acid utilisation as well as glycolytic reprogramming, suggesting that FOXK activity is strongly dependent on tissue and metabolic context.

1.5. Rationale and Scope of the Review

Current evidence supports FOXK1 and FOXK2 as important transcriptional regulators positioned downstream of insulin-associated signalling and linked to metabolic, mitochondrial, proliferative, and chromatin-associated processes. Their insulin-dependent nuclear response differs from the canonical FOXO1 response, providing a mechanistic framework for coordinated repression and activation of distinct transcriptional programmes [4,9].

Several questions nevertheless remain unresolved. First, the relative contributions of FOXK1 and FOXK2 to individual metabolic pathways remain incompletely defined. Although the proteins share conserved structural features and overlapping biological functions, their transcriptional effects can vary according to cellular context, tissue, interacting proteins, and metabolic state [7,9,11].

Second, the molecular hierarchy linking AKT, mTOR, GSK3, phosphorylation, and nuclear transport requires further clarification. Although pathway-level evidence supports AKT–mTOR-dependent nuclear accumulation and GSK3-dependent cytoplasmic retention, the contribution of individual phosphorylation events and intermediary proteins remains incompletely resolved [4].

Third, FOXK proteins function within broader chromatin regulatory networks. FOXK1/2 interact with chromatin-associated proteins and transcriptional regulators, including SIN3A-associated complexes and BAP1–HCF-1-containing complexes. These interactions indicate that FOXK-dependent transcription is determined not only by signalling-dependent nuclear localisation but also by the composition and activity of local chromatin regulatory complexes [8].

Accordingly, this review examines the molecular architecture and regulatory biology of FOXK1 and FOXK2, with emphasis on their integration into insulin–IR–IRS–PI3K–AKT–mTOR and GSK3 signalling. We further evaluate their relationship with FOXO1, regulation of glycolytic, lipid, and mitochondrial metabolism, control of proliferation and survival, and interactions with chromatin regulatory machinery. Particular emphasis is placed on distinguishing experimentally established mechanisms from associations and hypotheses that require validation in physiological and human disease settings.

2. Molecular Architecture of FOXK1 and FOXK2

2.1. FOXK1 and FOXK2 Gene and Protein Organisation

FOXK1 and FOXK2 are highly conserved members of the forkhead box (FOX) transcription-factor family. In humans, FOXK1 is located on chromosome 7p22, whereas FOXK2 is located on chromosome 17q25.3. The canonical human FOXK1 protein comprises 733 amino acids, while FOXK2 has multiple transcript isoforms, with the principal annotated isoform comprising approximately 660 amino acids [7,12]. Both proteins contain a conserved forkhead (FH) DNA-binding domain and a forkhead-associated (FHA) domain, a combination that distinguishes the FOXK subfamily from many other FOX proteins [13,14].

Beyond these conserved domains, FOXK proteins contain regulatory regions that determine subcellular localisation, transcriptional activity, and interactions with chromatin-associated proteins. FOXK1 possesses an N-terminal SIN3-interacting domain (SID), particularly within residues 1–40, that mediates interaction with SIN3 proteins and contributes to transcriptional repression [15]. FOXK1 also contains an FHA domain capable of recognising phosphorylated protein motifs, thereby providing a molecular interface between signalling-dependent phosphorylation and transcriptional regulation [16].

The structural similarity of FOXK1 and FOXK2 provides a basis for partially overlapping functions. Both proteins can bind regulatory chromatin regions and participate in transcriptional programmes controlling metabolism, proliferation, autophagy, and cellular adaptation. Nevertheless, structural similarity does not imply complete functional redundancy. Differences in expression, post-translational modification, interacting proteins, and chromatin environment can generate distinct transcriptional outputs [13,14].

2.2. Forkhead DNA-Binding Domain

The forkhead domain is a conserved winged-helix DNA-binding module that mediates sequence-specific recognition of regulatory DNA. In FOXK1 and FOXK2, this domain enables association with promoters and enhancers and provides the principal DNA-recognition function required for transcriptional regulation [13,14].

Genome-wide studies have established FOXK1 as a broad chromatin-associated transcription factor in the liver. Hepatic ChIP-seq identified FOXK1 occupancy at the proximal promoters and enhancers of more than 4000 genes. These genes included regulators of cell-cycle progression, autophagy, steroid biosynthesis, glucose metabolism, and mitochondrial function. Importantly, insulin increased FOXK1 chromatin association at approximately 75% of FOXK1-bound loci, demonstrating that insulin regulates FOXK1 not only through subcellular localisation but also through its association with genomic regulatory elements [9].

FOXK1 binding is enriched at a characteristic TGTTTAC DNA motif, although chromatin occupancy is influenced by the local regulatory environment and cooperating transcription factors [9]. FOXK1 also shows partial overlap with FOXO1 at selected hepatic loci, supporting functional integration between these two Forkhead proteins in insulin-responsive transcription [9].

FOXK1 and FOXK2 can occupy overlapping genomic regions, consistent with their conserved DNA-binding domains. However, shared occupancy should not be interpreted as equivalent transcriptional activity. Recent evidence indicates that FOXK1 and FOXK2 can produce different transcriptional outcomes despite recruitment to similar regulatory regions, suggesting that cofactor availability and post-translational modification contribute to factor-specific activity [14].

2.3. Forkhead-Associated (FHA) Domain

The FHA domain is a phosphoprotein-recognition module that preferentially recognises phosphorylated threonine-containing sequences. FOXK1 and FOXK2 are distinctive among FOX transcription factors because they combine an FHA domain with a forkhead DNA-binding domain [13,14].

The FHA domain provides a direct molecular connection between phosphorylation-dependent signalling and transcriptional regulation. One well-characterised example is the interaction of FOXK1 and FOXK2 with BRCA1-associated protein 1 (BAP1), a histone H2AK119 ubiquitin deubiquitinase. Both FOXK proteins use their FHA domains to recognise a phosphorylated threonine-containing region of BAP1, thereby facilitating recruitment of BAP1-containing chromatin regulatory complexes [8,14].

This interaction demonstrates that FOXK proteins do not function solely through sequence-specific DNA recognition. Their FHA domains enable them to sense the phosphorylation status of regulatory proteins and recruit chromatin-modifying machinery to selected genomic loci. Consequently, FOXK-dependent transcription can be determined by both DNA occupancy and the availability of phosphorylated protein partners.

An additional layer of regulation is provided by nutrient-sensitive O-GlcNAcylation. Recent experimental evidence demonstrated that FOXK1, but not FOXK2, undergoes O-GlcNAcylation and that this modification increases during the G1/S transition. FOXK1 O-GlcNAcylation enhances BAP1 recruitment to E2F-regulated promoters and enhancers and promotes transcription of E2F target genes. Loss of FOXK1 O-GlcNAcylation reduces BAP1 recruitment, increases the repressive histone mark H2AK119ub, and decreases H3K4me1 at affected regulatory regions [14]. These findings provide a direct molecular link between nutrient-sensitive post-translational modification, chromatin regulation, and cell-cycle control.

2.4. Nuclear–Cytoplasmic Localisation

Subcellular localisation is an important regulatory mechanism for FOXK1 and contributes to the translation of extracellular signals into transcriptional responses. Under basal conditions, FOXK1 is distributed between nuclear and cytoplasmic compartments. Insulin stimulation promotes redistribution of FOXK1 towards the nucleus and chromatin [17].

This process is closely linked to mTORC1 and GSK3 signalling. mTORC1 activity suppresses GSK3-dependent FOXK1 phosphorylation, whereas inhibition of mTORC1 increases FOXK1 phosphorylation. Phosphorylated FOXK1 associates with 14-3-3 proteins, resulting in reduced DNA binding and nuclear exclusion. Thus, mTORC1 promotes FOXK1 transcriptional activity, at least in part, by restraining GSK3-dependent phosphorylation [17].

Insulin-responsive FOXK1 localisation is consistent with this signalling architecture. Inhibition of PI3K or AKT attenuates insulin-induced FOXK1 nuclear accumulation, while mTOR inhibition interferes with the nuclear response. Conversely, inhibition or depletion of GSK3 promotes FOXK1 nuclear accumulation [4,17]. These findings position FOXK1 downstream of an AKT–mTOR–GSK3 regulatory module.

The available evidence for this phosphorylation–14-3-3 mechanism is considerably stronger for FOXK1 than for FOXK2. FOXK2 also responds to insulin and exhibits substantial nuclear localisation, but the precise molecular determinants governing its subcellular distribution remain less completely defined [4]. Therefore, mechanisms established for FOXK1 should not automatically be extrapolated to FOXK2.

2.5. Functional Overlap and Context-Dependent Specificity

FOXK1 and FOXK2 share conserved structural features and participate in several common biological processes, including metabolic regulation, cell proliferation, autophagy, mitochondrial function, and transcriptional responses to nutrient availability [4,13,14]. In myogenic progenitor cells, FOXK1 recruits SIN3–SDS3-associated repression machinery through its FHA domain and contributes to transcriptional repression of cell-cycle inhibitory programmes [15]. FOXK proteins have also been linked to chromatin-associated regulatory complexes containing SIN3A, HDAC3, BAP1, and other transcriptional cofactors [8,14,16].

Despite these shared properties, FOXK1 and FOXK2 are not functionally interchangeable. FOXK1 has been particularly implicated in hepatic insulin responses, metabolic reprogramming, cell-cycle regulation, and E2F-dependent transcription [4,9,14,17]. FOXK2 participates in overlapping transcriptional networks but can exhibit distinct biological effects depending on cellular and disease context. Recent work directly demonstrated that FOXK1, but not FOXK2, promoted E2F target-gene expression through O-GlcNAcylation-dependent recruitment of BAP1 [14].

Functional redundancy is nevertheless evident in selected settings. Experimental depletion of FOXK1 and FOXK2 in hepatic cells produces overlapping metabolic and mitochondrial phenotypes, with combined depletion producing more pronounced effects than depletion of either factor alone [4]. Such findings indicate partial compensation between the proteins while retaining the possibility of factor-specific functions.

Cellular context therefore represents a major determinant of FOXK activity. At one locus, FOXK proteins can participate in transcriptional repression through SIN3-associated complexes, at another, they can support transcriptional activation through BAP1, E2F, AP1, or metabolic regulatory networks [8,14,16]. The functional state of FOXK1/2 is consequently determined by the integration of DNA occupancy, post-translational modification, signalling activity, chromatin accessibility, and interacting cofactors.

Taken together, the molecular architecture of FOXK1 and FOXK2 provides a mechanistic framework for their role as transcriptional integrators. Their forkhead domains provide DNA recognition, FHA domains enable phosphoprotein-dependent interactions, and regulatory regions determine interactions with transcriptional and chromatin-associated complexes. These structural properties allow FOXK proteins to connect insulin, nutrient, and growth signalling with longer-term transcriptional control of metabolism, proliferation, and cellular adaptation.

3. Insulin-Dependent Regulation of FOXK1 and FOXK2

3.1. Insulin Receptor–IRS–PI3K–AKT Signalling

Regulation of FOXK1 and FOXK2 by insulin is initiated within the canonical insulin-receptor signalling network. Insulin binding activates the intrinsic tyrosine kinase activity of the insulin receptor (IR), resulting in phosphorylation of insulin receptor substrates (IRS proteins) and recruitment of phosphoinositide 3-kinase (PI3K). PI3K-generated phosphatidylinositol-3,4,5-trisphosphate promotes activation of PDK1 and AKT, which

subsequently regulates multiple metabolic and transcriptional effectors, including mTOR, GSK3, and FOXO proteins [18,19].

Proteomic analysis of receptor-associated protein complexes identified FOXK1 as a ligand-dependent interactor of IR- and IGF1R-containing signalling complexes. This finding implicated FOXK1 as a component of receptor-associated signalling rather than simply as a secondary metabolic target [4]. Importantly, the association does not establish FOXK1 as a direct kinase substrate of IR or IGF1R.

Functional studies demonstrated that insulin-induced FOXK1 nuclear accumulation is strongly dependent on PI3K–AKT signalling. Pharmacological inhibition of PI3K or AKT retained FOXK1 in the cytoplasm after insulin stimulation, whereas MEK/ERK inhibition produced comparatively limited effects. Similar FOXK1 nuclear redistribution was observed following IGF1 stimulation, indicating that FOXK1 can respond to signals transmitted through both IR and IGF1R [4].

FOXK1 and FOXK2 may also modulate the signalling network upstream of their transcriptional effects. Depletion of either FOXK1 or FOXK2 increased insulin-stimulated phosphorylation of AKT, ERK1/2, and ribosomal S6 protein, whereas FOXK overexpression reduced ERK1/2 and S6 phosphorylation. These findings indicate that FOXK1/2 can influence the magnitude of insulin-associated signalling, although the molecular basis of this feedback regulation remains incompletely defined [4].

3.2. AKT–mTOR-Dependent FOXK Nuclear Accumulation

The AKT–mTOR pathway represents a major regulatory axis controlling insulin-dependent FOXK nuclear accumulation. Insulin stimulation activates AKT and downstream mTOR signalling, whereas pharmacological inhibition of mTOR with rapamycin prevents insulin-induced nuclear accumulation of FOXK1. These findings place mTOR signalling downstream of PI3K–AKT and upstream of FOXK1 nuclear redistribution [4].

Independent mechanistic studies established a direct relationship between mTORC1 activity and FOXK1 phosphorylation. mTORC1 suppresses GSK3-dependent FOXK1 phosphorylation, when mTORC1 activity is reduced, FOXK1 phosphorylation increases, promoting 14-3-3 association, reducing DNA binding, and favouring nuclear exclusion [17]. Thus, mTORC1 can enhance FOXK1 transcriptional activity by limiting a phosphorylation-dependent mechanism that restricts nuclear access.

This signalling architecture provides a molecular link between nutrient sensing and transcriptional regulation. Insulin activates AKT, which inhibits GSK3, while AKT-dependent signalling also promotes mTORC1 activity. The combined reduction in GSK3-mediated FOXK phosphorylation and activation of mTORC1 therefore favours nuclear FOXK activity [4,17].

Once nuclear, FOXK proteins regulate transcriptional programmes associated with cellular metabolism, mitochondrial function, proliferation, and survival [4,11,17]. Consequently, the AKT–mTOR–FOXK axis extends insulin signalling from receptor-proximal kinase activation to sustained transcriptional adaptation.

3.3. GSK3-Dependent Cytoplasmic Retention

GSK3α and GSK3β provide an opposing regulatory mechanism that contributes to basal cytoplasmic retention of FOXK proteins. Phosphoproteomic analysis identified insulin-responsive phosphorylation clusters in FOXK1 and FOXK2 containing S-X-X-X-S/T motifs compatible with GSK3 substrate recognition [4].

Inhibition of GSK3 with CHIR99021 produced marked nuclear accumulation of FOXK1 and FOXK2 even in the absence of insulin. Similarly, combined depletion of GSK3α and GSK3β increased nuclear FOXK1 localisation, whereas re-expression of GSK3β partially restored cytoplasmic localisation [4]. These findings establish GSK3 activity as an important determinant of FOXK subcellular distribution.

Insulin therefore produces an opposing regulatory response: AKT inhibits GSK3 while promoting mTOR signalling, thereby reducing the phosphorylation-dependent constraints on FOXK nuclear accumulation [4,17].

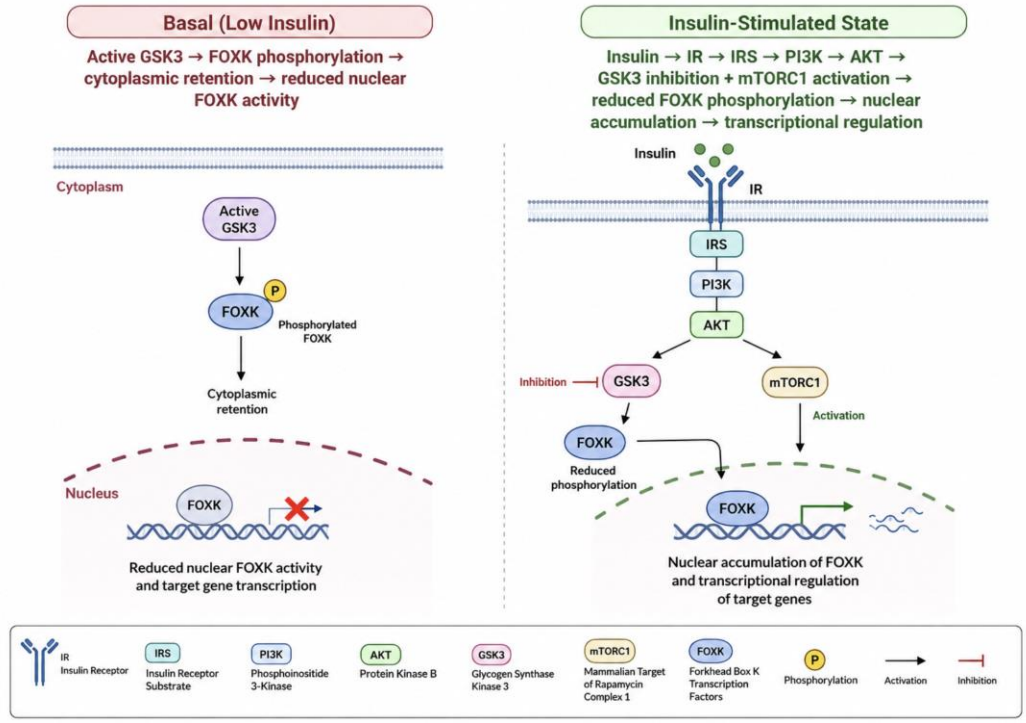

Figure 1: The Experimentally Supported Signalling Framework Can Be Summarised as: In diagramatic form .This Model is most Firmly Established for FOXK1, although GSK3 Inhibition also Promotes Nuclear Accumulation of FOXK2. The Molecular Details Underlying FOXK2 Localisation Remain Less Completely Characterised [4]. in shown figure 1

3.4. Phosphorylation-Dependent Regulation

Phosphorylation represents an important regulatory layer controlling FOXK localisation and protein interactions. Global phosphoproteomic analysis identified receptor- and ligand-responsive phosphorylation clusters in both FOXK1 and FOXK2. FOXK1 contained an N-terminal cluster involving Ser225, Ser229, and Thr233, together with C-terminal clusters involving Ser402/Ser406 and Ser454/Ser458. FOXK2 contained a corresponding C-terminal cluster involving Ser415/Ser419 [4].

These phosphorylation responses were heterogeneous. The N-terminal FOXK1 cluster increased following insulin or IGF1 stimulation, whereas several C-terminal sites showed reduced phosphorylation following receptor activation. Thus, insulin appears to remodel the FOXK phosphorylation landscape rather than producing a uniform increase or decrease in phosphorylation [4].

The C-terminal FOXK1 sites are particularly relevant because their S-X-X-X-S/T sequence is compatible with GSK3-dependent phosphorylation. Mutation of selected sites, including Ser402/Ser406 and Ser454/Ser458, reduced cytoplasmic localisation and increased nuclear accumulation of FOXK1 [4]. These experiments provide functional evidence that phosphorylation at these residues contributes to FOXK1 subcellular regulation.

The mTORC1 study further demonstrated that suppression of mTORC1 increases GSK3-dependent FOXK1 phosphorylation, promotes 14-3-3 binding, reduces DNA association, and favours nuclear exclusion [17]. Together, these findings establish phosphorylation as a molecular interface between mTORC1 and GSK3 signalling in FOXK1 regulation.

However, phosphorylation is unlikely to regulate FOXK proteins solely through subcellular localisation. Because phosphorylation can modify protein–protein interactions and transcriptional-complex assembly, insulin-dependent changes in FOXK phosphorylation may also alter DNA binding and recruitment of regulatory cofactors. The relative contribution of individual phosphorylation sites to these functions remains an important area for further investigation.

3.5. FOXK1/2–FOXO1 Reciprocal Localisation

A defining feature of insulin-dependent FOXK regulation is the opposing subcellular response of FOXK1/2 and FOXO1. In the basal state, FOXO1 is predominantly nuclear, whereas FOXK1 is distributed between cytoplasmic and nuclear compartments. Following insulin stimulation, AKT-dependent FOXO1 phosphorylation promotes its nuclear exclusion, while insulin promotes FOXK1 and FOXK2 nuclear accumulation [4].

This reciprocal response provides a potential mechanism for coordinated transcriptional reprogramming. Insulin can simultaneously suppress fasting-associated FOXO1 activity and increase the nuclear availability of FOXK proteins, thereby allowing distinct transcriptional programmes to respond to the

same hormonal signal.

The relationship between FOXK1 and FOXO1 is nevertheless more complex than simple reciprocal localisation. FOXK1 binds insulin-responsive hepatic regulatory regions, including the PCK1 promoter, and contributes to regulation of hepatic glucose production [10]. Genome-wide analysis subsequently demonstrated that FOXK1 occupies more than 4000 hepatic promoters and enhancers, with insulin increasing FOXK1 binding at approximately 75% of these sites. Some FOXK1-bound genes are also occupied by FOXO1, supporting functional integration between the two transcription factors [9].

Experimental depletion of FOXK1 in primary hepatocytes reduced glucose production and impaired insulin-mediated suppression of glucose output, identifying FOXK1 as a functional contributor to insulin-regulated hepatic glucose metabolism [10]. The subsequent hepatic chromatin study further showed that FOXK1 and FOXO1 can participate together in regulation of genes involved in mitochondrial translation, metabolism, and cellular adaptation [9].

These observations support a model in which FOXK1 and FOXO1 occupy complementary but partially overlapping positions within the insulin-responsive transcriptional network. FOXO1 remains a principal regulator of fasting-associated hepatic transcription, whereas FOXK1 contributes to a broader insulin-responsive network encompassing glucose metabolism, lipid metabolism, mitochondrial function, cell-cycle regulation, and other cellular programmes [9,10].

The reciprocal FOXK–FOXO1 response therefore represents more than a simple exchange of nuclear and cytoplasmic localisation. It reflects coordinated redistribution of transcriptional control in response to insulin, in which changes in nuclear abundance, phosphorylation, chromatin occupancy, and cofactor interactions collectively determine gene expression.
Overall, insulin-dependent regulation of FOXK1 and FOXK2 can be conceptualised as an integrated signalling axis linking receptor activation to transcriptional adaptation:
IR/IGF1R → IRS → PI3K → AKT → mTORC1/GSK3 → FOXK localisation and phosphorylation → chromatin engagement → metabolic and cellular transcriptional responses.

This axis complements the canonical AKT–FOXO1 pathway and provides an expanded framework for understanding how insulin coordinates repression of fasting-associated programmes with activation of metabolic, mitochondrial, proliferative, and survival-associated transcription.

4. FOXK1/FOXK2 as Transcriptional Integrators of Insulin Signalling

FOXK1 and FOXK2 occupy an emerging position between insulin-dependent kinase signalling and transcriptional regulation. Unlike FOXO1, whose transcriptional activity is generally attenuated by insulin-induced nuclear exclusion, FOXK1 and FOXK2 undergo insulin-dependent nuclear accumulation. This opposing response suggests that insulin can simultaneously repress fasting-associated FOXO transcription and engage FOXK-dependent transcriptional programmes. Experimental studies have linked FOXK proteins to metabolic regulation, mitochondrial function, proliferation, survival, and cellular adaptation [4].

The designation of FOXK1/FOXK2 as transcriptional integrators is supported by several complementary observations. Insulin-dependent FOXK nuclear accumulation requires PI3K–AKT–mTOR signalling, whereas GSK3 activity contributes to basal cytoplasmic retention [4,17]. Genome-wide hepatic chromatin profiling further demonstrated that FOXK1 occupies more than 4000 promoters and enhancers and that insulin increases FOXK1 occupancy at approximately 75% of these sites [9]. In addition, depletion of FOXK1 or FOXK2 alters insulin-stimulated AKT, ERK1/2, and S6 phosphorylation, suggesting that FOXK proteins can modulate the signalling network in addition to responding to it [4]. Collectively, these observations support a model in which FOXK proteins convert insulin- and nutrient-dependent signalling into longer-lasting transcriptional responses.

4.1. Chromatin Binding and Promoter Regulation

FOXK-dependent transcription requires sequence-specific DNA recognition together with recruitment of transcriptional and chromatin-associated cofactors. FOXK1 and FOXK2 contain conserved forkhead DNA-binding domains and FHA domains that facilitate protein interactions, allowing their transcriptional effects to vary according to cellular context and associated regulatory complexes [20,21].

Initial evidence linking FOXK1 to insulin-responsive hepatic transcription was obtained using DNA-affinity purification and transcription-factor profiling. Wang et al. identified FOXK1 as an insulin-responsive regulator associated with regulatory regions of Pck1 and G6pc, genes involved in hepatic glucose production. FOXK1 depletion reduced hepatocyte glucose production and impaired the suppressive effect of insulin on glucose output, supporting a functional role for FOXK1 in hepatic insulin action [10].

Genome-wide chromatin analysis subsequently provided broader evidence for FOXK1-dependent transcriptional regulation in liver. Allu et al. showed that FOXK1 occupies proximal promoters and enhancers associated with more than 4000 genes and that insulin enhances FOXK1 occupancy at approximately 75% of these sites. FOXK1-bound genes were enriched in pathways related to cell-cycle regulation, senescence, steroid biosynthesis, autophagy, glucose metabolism, lipid metabolism, and mitochondrial function [9]. The predominant FOXK1-associated DNA motif was TGTTTAC, consistent with sequence-specific recognition through the forkhead domain [9].

The hepatic FOXK1 regulatory network also includes genes associated with cellular and metabolic processes that extend beyond classical glucose metabolism. FOXK1 occupancy was observed at regulatory regions associated with LARS1 and TIMM22,

implicating FOXK1 in pathways related to rRNA processing, mitochondrial protein import, and cell-cycle regulation [9]. These findings indicate that FOXK1 acts within a broad transcriptional network rather than controlling a restricted set of metabolic genes.

FOXK1 also appears to operate in combination with other transcription factors. Motif enrichment analyses identified hepatocyte nuclear factor 4α (HNF4α) among potential regulatory partners, while subsets of FOXK1-bound genes were also occupied by FOXO1 [9]. This overlapping occupancy supports a model of combinatorial transcriptional control in which insulin-responsive gene expression is determined by interactions among multiple transcription factors rather than by FOXK1 alone.

The FOXK1–FOXO1 relationship is particularly relevant to hepatic insulin action. Insulin promotes FOXO1 nuclear exclusion while increasing FOXK1 nuclear accumulation, thereby altering the relative availability of these two forkhead regulators [4,9]. FOXK1 and FOXO1 may consequently contribute to different, but partially overlapping, transcriptional responses during the transition from fasting to insulin-replete conditions. Importantly, the extent to which they cooperate or compete is likely to depend on the target gene and cellular state.

mTORC1 provides an additional regulatory layer. Reduced mTORC1 activity increases GSK3-dependent FOXK1 phosphorylation, promotes 14-3-3 association, decreases DNA binding, and favours nuclear exclusion [17]. Conversely, mTORC1 activity suppresses this inhibitory phosphorylation mechanism and promotes FOXK1-dependent metabolic transcription [17]. These observations position FOXK1 downstream of nutrient-sensitive mTORC1 signalling and provide a mechanistic link between kinase activity, nuclear localisation, chromatin engagement, and metabolic gene regulation.

Overall, FOXK1 should therefore be viewed as a signal-responsive transcriptional regulator whose activity is determined by the integrated effects of phosphorylation, subcellular localisation, DNA occupancy, and interaction with transcriptional cofactors. Evidence for an equivalent genome-wide insulin-responsive chromatin mechanism for FOXK2 remains considerably more limited.

4.2. Transcriptional Feedback on Insulin Signalling

FOXK1 and FOXK2 are not exclusively passive targets of insulin signalling. Experimental studies indicate that perturbation of either factor can alter the magnitude of downstream insulin-responsive kinase activity [4].

Sakaguchi et al. reported that FOXK1 or FOXK2 depletion did not substantially alter insulin receptor expression, receptor tyrosine phosphorylation, or IRS1 phosphorylation. In contrast, insulin-stimulated phosphorylation of AKT, ERK1/2, and ribosomal S6 protein increased following FOXK1 or FOXK2 depletion. Conversely, FOXK1/2 overexpression reduced selected downstream signalling responses [4]. These findings suggest that FOXK proteins can participate in feedback modulation downstream of receptor activation.

Transcriptomic analysis further identified changes in genes involved in intracellular signalling following FOXK depletion, including Dusp5, Ppp1ca, Spry4, and other regulatory genes [4]. Because DUSP5 regulates ERK signalling and PP1 catalytic subunits participate in protein dephosphorylation, altered expression of such genes provides a plausible mechanism through which FOXK-dependent transcription could influence signalling intensity. However, the causal contribution of each individual gene to the observed kinase phenotype has not been fully established.

The relationship can therefore be represented as a proposed feedback architecture:
Insulin → IR/IRS → PI3K–AKT → mTOR → FOXK1/FOXK2 nuclear accumulation → transcription of signalling regulators → modulation of AKT/ERK/S6 activity.
This model should be regarded as a feedback-modulatory framework rather than a fully resolved linear feedback pathway. It is supported experimentally at the level of FOXK perturbation and downstream kinase phosphorylation, whereas the precise transcriptional intermediates responsible for the feedback remain incompletely defined.

FOXK-dependent modulation of AKT activity may also influence FOXO1. FOXK depletion was associated with altered insulin-dependent FOXO1 cytoplasmic redistribution, consistent with changes in AKT signalling [4]. Thus, FOXK and FOXO1 may be interconnected at two levels: both respond to the insulin–AKT pathway, while FOXK-dependent feedback may subsequently influence the kinase activity that controls FOXO1 localisation.

4.3. Regulation of Cell-Cycle Programs

Beyond metabolic regulation, FOXK proteins participate in transcriptional programmes controlling cellular proliferation and cell-cycle progression. This function is particularly relevant to insulin and mTOR signalling because these pathways simultaneously regulate nutrient utilisation, biosynthetic activity, cell growth, and proliferation.

In hepatocytes, combined FOXK1/FOXK2 depletion produced broad transcriptional changes involving proliferation-associated genes and was associated with reduced cellular proliferation [4]. Genome-wide hepatic FOXK1 occupancy further revealed enrichment at genes involved in cell-cycle regulation and cellular senescence [9]. These findings provide evidence that FOXK-dependent transcription is linked to proliferative programmes in metabolic tissues.

Independent cancer-model studies have provided more direct mechanistic evidence for FOXK1. In ovarian cancer cells, FOXK1 promoted proliferation and G1/S progression through transcriptional repression of the cyclin-dependent kinase inhibitor p21/CDKN1A [22]. In glioblastoma models, FOXK1 promoted proliferation and increased the S-phase population, while chromatin

and reporter assays supported direct activation of SNAI1/Snail transcription [23]. These observations demonstrate that FOXK1 can regulate cell-cycle and malignant phenotypes, although they should not be interpreted as evidence that these mechanisms are universally operative downstream of insulin.

FOXK2 also contributes to proliferation. Van der Heide et al. reported that FOXK2 depletion reduced BrdU incorporation and histone H3 phosphorylation, consistent with impaired DNA synthesis and proliferative activity. FOXK2 depletion also increased expression of Gadd45a and Gadd45g, indicating activation of stress-associated transcriptional responses [24].

More recently, FOXK1 has been directly linked to the E2F transcriptional network. Ahmed et al. demonstrated that FOXK1 promotes expression of E2F target genes and that FOXK1 O-GlcNAcylation is required for efficient recruitment of BAP1 to E2F-regulated chromatin. Loss of FOXK1 O-GlcNAcylation reduced E2F-associated transcription, cellular proliferation, transformation, and tumour growth [14]. This mechanism provides an important example of how nutrient-sensitive post-translational modification can couple FOXK1 activity to proliferative transcription.

Collectively, these findings suggest that FOXK proteins can connect nutrient and growth-factor signalling with transcriptional programmes governing cell-cycle progression. However, the E2F–BAP1–O-GlcNAc mechanism is currently best established for FOXK1 rather than FOXK2 [14].

4.4. Regulation of Apoptosis and Cell Survival

FOXK proteins also participate in transcriptional programmes associated with cellular survival, although the experimental evidence differs between FOXK1 and FOXK2.

In the hepatic model, combined FOXK1/FOXK2 depletion altered apoptosis-associated transcription and was accompanied by reduced proliferation and impaired mitochondrial metabolic function [4]. The phenotype was particularly pronounced after combined depletion, suggesting functional compensation between the two factors. These findings support an indirect contribution of FOXK proteins to cellular survival through maintenance of metabolic and mitochondrial homeostasis.

More direct evidence exists for FOXK2. Van der Heide et al. demonstrated that FOXK2 depletion increased caspase-3 activity and propidium iodide-positive cell death under growth-factor-deprived conditions. FOXK2 depletion also increased expression of the pro-apoptotic BCL2-family members PUMA and NOXA [24]. These findings support a role for FOXK2 in maintaining a transcriptional state compatible with cellular survival.

The same study identified an interaction between FOXK2 and mTOR-dependent signalling. FOXK2 depletion increased phosphorylation of the mTOR effector p70S6K, while rapamycin potentiated the proliferation defect caused by FOXK2 loss [24]. These findings suggest that mTOR signalling can partially compensate for FOXK2 deficiency, although the molecular relationship is more appropriately considered a functional interaction than a direct FOXK2–mTOR regulatory pathway.

For FOXK1, evidence for a direct anti-apoptotic role is less consistent. In an ovarian cancer model, FOXK1 promoted proliferation but did not significantly alter apoptosis [22]. Therefore, FOXK1-associated survival phenotypes should be interpreted cautiously and may reflect preservation of metabolic or proliferative fitness rather than direct suppression of apoptotic machinery.

The FOXK–BAP1 axis provides an additional chromatin-based mechanism relevant to proliferation and cellular transformation. FOXK1 can recruit BAP1 to regulatory chromatin, and nutrient-sensitive O-GlcNAcylation of FOXK1 enhances this interaction at E2F target genes [14]. This mechanism links metabolic state, chromatin regulation, and proliferative output, but it should not be generalized to FOXK2 without additional experimental evidence.

Overall, current evidence supports FOXK-dependent regulation of cellular survival through a combination of transcriptional, metabolic, mitochondrial, and chromatin mechanisms. The direct apoptotic role is more clearly established for FOXK2, whereas FOXK1 appears to exert stronger effects through metabolic fitness and proliferative transcriptional programmes.

4.5. FOXK1 Versus FOXK2

FOXK1 and FOXK2 share considerable structural similarity and overlapping biological functions, but available evidence indicates that they are not functionally interchangeable. Their relative contribution depends on tissue, cellular state, signalling environment, chromatin landscape, and availability of interacting cofactors.

4.5.1. FOXK1

FOXK1 has the strongest evidence for direct integration with hepatic insulin-responsive transcription. It occupies thousands of hepatic promoters and enhancers, with insulin increasing FOXK1 chromatin association at a large proportion of these sites [9]. FOXK1 also contributes to regulation of hepatic glucose production through association with insulin-responsive regulatory regions, including Pck1 and G6pc [10].

FOXK1 is additionally linked to mTORC1-dependent metabolic reprogramming. mTORC1 suppresses GSK3-dependent FOXK1 phosphorylation, thereby promoting FOXK1 nuclear activity and metabolic gene regulation [17]. FOXK1 also regulates proliferative transcriptional programmes, including p21-associated cell-cycle control and the E2F pathway [14,22].

Thus, the strongest experimentally supported FOXK1 functions include:

- hepatic insulin-responsive transcription,
- glucose and lipid metabolic regulation,

- mTORC1-dependent metabolic reprogramming,
- mitochondrial and cellular metabolic regulation,
- cell-cycle control, and
- E2F-associated proliferative transcription.

4.5.2. FOXK2

FOXK2 shares insulin responsiveness with FOXK1 but has a comparatively greater documented role in proliferation, survival, and transcriptional homeostasis. Sakaguchi et al. demonstrated that FOXK2 undergoes insulin-dependent nuclear accumulation and contributes to metabolic and mitochondrial regulation [4].

Independent functional studies showed that FOXK2 depletion reduces proliferation and increases apoptosis-associated phenotypes, including caspase-3 activation and induction of PUMA and NOXA [24]. FOXK2 therefore has particularly strong experimental support for roles in cellular proliferation and survival.

However, the evidence for FOXK2 as a genome-wide hepatic insulin-responsive transcriptional regulator is less extensive than that available for FOXK1. The large-scale hepatic ChIP-seq study was specifically focused on FOXK1 [20]. Therefore, statements describing more than 4000 insulin-responsive FOXK-bound genes should be attributed to FOXK1 rather than to FOXK1/FOXK2 collectively.

4.5.3. Shared Functions

Despite these differences, FOXK1 and FOXK2 display substantial functional overlap. Combined depletion produces stronger metabolic and transcriptional phenotypes than depletion of either factor alone, consistent with partial functional compensation [4]. Both proteins participate in:

- insulin-responsive transcription,
- mitochondrial metabolic regulation,
- glucose and lipid metabolism,
- cellular proliferation,
- survival-associated transcription,
- chromatin regulation, and
- nutrient-responsive cellular adaptation [4,9,24].

4.5.4. Distinct Functional Characteristics

Feature FOXK1 FOXK2
Insulin-responsive nuclear accumulation Strong experimental evidence Demonstrated
Basal localisation More cytoplasmic Relatively more nuclear
Hepatic insulin-responsive chromatin Strong evidence Limited comparative evidence
Hepatic glucose regulation Strong evidence Less well defined
mTORC1–GSK3 regulation Mechanistically established Less extensively defined
Mitochondrial metabolism Strong evidence Strong contributory evidence
Glycolytic/metabolic reprogramming Strong evidence Contributory
Cell-cycle regulation Strong evidence Strong evidence
E2F regulation Direct evidence Less established
O-GlcNAcylation–BAP1 mechanism Demonstrated Not established equivalently
Apoptosis/survival Mainly indirect/context dependent Stronger direct evidence
PUMA/NOXA regulation Not established Demonstrated
Functional redundancy Partial Partial Taken together, the available evidence supports a model in which FOXK1 and FOXK2 constitute a partially redundant but functionally specialized transcriptional pair. FOXK1 has particularly strong evidence as an insulin-responsive hepatic and mTOR-regulated transcriptional effector, whereas FOXK2 has prominent experimentally supported roles in proliferation and cellular survival [4,9,17,24].

Importantly, these distinctions should not be interpreted as absolute. Both proteins can contribute to overlapping metabolic and cellular programmes, and their relative functions are likely to vary with tissue, nutrient state, chromatin environment, and interacting proteins. Consequently, FOXK1 and FOXK2 should be analysed as related but distinct regulatory nodes rather than as interchangeable transcription factors.

From a disease perspective, persistent activation of insulin–PI3K–AKT–mTOR signalling and altered nutrient-sensitive post-translational regulation could potentially enhance FOXK-dependent transcription in pathological settings. The FOXK1 O-GlcNAc–BAP1–E2F pathway provides direct evidence that nutrient-sensitive FOXK regulation can promote oncogenic transcription and tumour growth [14]. However, the extent to which physiological insulin signalling drives this mechanism in human metabolic disease or cancer remains to be established. Thus, the current evidence supports FOXK1/FOXK2 as candidate transcriptional integrators of insulin, nutrient, and growth signalling, while several tissue-specific and disease-associated mechanisms remain under investigation.

5. FOXK1/FOXK2 and Metabolic Reprogramming

Metabolic reprogramming enables proliferating cells to adapt carbon and energy flux to increased biosynthetic and energetic requirements. FOXK1 and FOXK2 have emerged as transcriptional regulators that connect nutrient-responsive signalling with metabolic gene expression. Experimental studies indicate that FOXK1/2 promote glycolytic metabolism while influencing pyruvate utilisation, lipid metabolism and mitochondrial function. Their metabolic effects are context dependent and are influenced by cellular lineage, nutrient status and upstream signalling activity [25].

The metabolic phenotype associated with FOXK proteins is particularly relevant to cancer because alterations in glucose and lipid metabolism can support ATP generation, biosynthesis and cellular proliferation. Recent evidence in cervical cancer has further implicated FOXK2 in lipid metabolic remodelling. FOXK2 overexpression increased CPT1A expression and fatty-acid oxidation while reducing ACC1 and FASN expression. These changes were associated with activation of the mTOR/DRP1 axis and enhanced malignant phenotypes in experimental models

[26]. Importantly, this mechanism has so far been demonstrated directly in cervical cancer models and should not be extrapolated indiscriminately to other tumour types.

5.1. Regulation of Glycolysis

FOXK1 and FOXK2 directly contribute to transcriptional regulation of the glycolytic programme. Experimental manipulation of either factor alters the abundance of multiple glycolytic components, including HK2, PFK, PK and LDHA, indicating coordinated regulation of glycolytic flux rather than control of an isolated enzymatic step [11].

FOXK1 also functions as an effector of mTORC1-dependent metabolic regulation. Suppression of mTORC1 increases GSK3-dependent FOXK1 phosphorylation, promotes 14-3-3 association and reduces FOXK1 DNA binding and nuclear localisation. Conversely, active mTORC1 restrains this inhibitory phosphorylation and permits FOXK1-dependent transcription of genes involved in glycolysis and anabolic metabolism, partly through regulation of HIF1A [17].

These findings establish a mechanistic connection between nutrient-sensitive mTORC1 signalling and transcriptional control of glucose metabolism. However, the mTORC1–GSK3 mechanism is most directly established for FOXK1, equivalent mechanistic evidence for FOXK2 remains less extensive [17].

5.2. Aerobic Glycolysis and Lactate Production

Aerobic glycolysis is one of the best-characterised metabolic functions of FOXK1 and FOXK2. Sukonina et al. demonstrated that increased FOXK1 or FOXK2 activity enhances expression of glycolytic enzymes and increases lactate production despite the presence of oxygen [11]. Both gain- and loss-of-function experiments, including studies in primary human cells and experimental models, supported a role for FOXK proteins in regulating this metabolic phenotype [11].

FOXK1/2 additionally influence the fate of glycolytically generated pyruvate. Increased expression of PDK1 and PDK4, together with reduced activity of pyruvate dehydrogenase phosphatase 1, increases inhibitory phosphorylation of the E1α component of the pyruvate dehydrogenase complex. This restricts conversion of pyruvate to acetyl-CoA and favours its reduction to lactate [11].

Thus, FOXK1/2 coordinate glycolysis with suppression of mitochondrial pyruvate oxidation. This arrangement can support rapid glucose utilisation and maintain carbon availability for biosynthetic pathways. Nevertheless, FOXK-dependent aerobic glycolysis should be interpreted as a metabolic adaptation rather than evidence that mitochondrial oxidative metabolism is universally suppressed.

5.3. Fatty-Acid Oxidation

FOXK1 and FOXK2 also influence lipid metabolism. In hepatic insulin-responsive models, depletion of FOXK1/2 altered transcriptional programmes associated with lipid metabolism and mitochondrial fatty-acid utilisation and was accompanied by changes in mitochondrial fatty-acid oxidation [25]. These findings place FOXK proteins within the transcriptional network connecting insulin signalling to lipid utilisation.

More direct evidence for FOXK2-dependent fatty-acid metabolic regulation has recently emerged from cervical cancer models. Liao et al. showed that FOXK2 overexpression increased CPT1A expression and fatty-acid oxidation, whereas FOXK2 depletion produced the opposite phenotype. FOXK2 overexpression simultaneously reduced expression of ACC1 and FASN, indicating a shift towards fatty-acid utilisation and away from de novo lipogenesis [26].

The same study linked FOXK2 to the mTOR/DRP1 signalling axis. FOXK2 manipulation altered mTOR phosphorylation and DRP1 expression, while pharmacological modulation of mTOR or fatty-acid oxidation partially modified the metabolic phenotype. These findings support a FOXK2–mTOR/DRP1 axis in cervical cancer-associated lipid metabolic reprogramming [26]. However, the precise biochemical relationship between FOXK2, mTOR and DRP1 requires further validation, and the findings should presently be regarded as disease- and model-specific.

5.4. Pyruvate Oxidation

Pyruvate constitutes a major metabolic branch point between glycolysis and mitochondrial oxidative metabolism. FOXK1 and FOXK2 influence this transition through regulation of the pyruvate dehydrogenase system. Increased FOXK1/2 activity promotes PDK1 and PDK4 expression and reduces pyruvate dehydrogenase activity through inhibitory phosphorylation of the complex. Consequently, mitochondrial oxidation of pyruvate decreases and lactate production increases [11].

This mechanism positions FOXK1/2 as transcriptional regulators of the glycolysis–oxidative metabolism balance. Increased FOXK activity favours glycolytic disposal of glucose-derived pyruvate, whereas FOXK suppression permits greater mitochondrial utilisation of pyruvate [11].

The biological significance of this metabolic switch is context dependent. Rapidly proliferating cells may benefit from increased glycolytic flux because it can simultaneously provide ATP and intermediates for biosynthetic pathways. However, whether FOXK-dependent control of pyruvate oxidation is a dominant metabolic mechanism in individual tumour types remains to be established experimentally.

5.5. Metabolic Flexibility

An important feature of FOXK biology is the ability to coordinate several metabolic pathways rather than regulate a single substrate pathway. Insulin promotes FOXK1/2 nuclear accumulation through an AKT–mTOR-dependent mechanism, whereas GSK3 contributes to their cytoplasmic retention under basal conditions [25]. This provides a signalling mechanism through which hormonal and nutrient availability can alter FOXK-dependent

transcription.

Downstream of this signalling response, FOXK1/2 influence glycolysis, pyruvate utilisation, lipid metabolism and mitochondrial function [11,25]. Collectively, these effects suggest that FOXK proteins contribute to metabolic flexibility by coordinating alternative routes of carbon utilisation according to cellular requirements.

Nevertheless, FOXK1 and FOXK2 should not be assumed to be metabolically identical. Aerobic glycolysis is experimentally established for both factors, whereas the mTORC1–GSK3–HIF1A mechanism is particularly well characterised for FOXK1 [11,17]. Conversely, direct regulation of fatty-acid oxidation through the mTOR/DRP1 axis has recently been demonstrated for FOXK2 in cervical cancer [26]. These differences support a model of partial functional overlap combined with context-dependent specialization.

Overall, the available evidence positions FOXK1 and FOXK2 as nutrient-responsive transcriptional regulators that integrate insulin-, mTOR- and GSK3-associated signalling with metabolic gene expression. Their ability to coordinate glycolysis, pyruvate disposition, lipid utilisation and mitochondrial metabolism provides a mechanistic basis for adaptation to changing nutrient and growth conditions. In malignancy, these physiological functions may be co-opted to support metabolic plasticity and proliferation, but the extent to which FOXK-dependent metabolic programmes are causal drivers rather than consequences of malignant transformation remains an important area for investigation.

6. FOXK1/FOXK2 and Mitochondrial Function

Mitochondria are central to the metabolic programs regulated by FOXK1 and FOXK2 because they integrate fatty-acid oxidation, pyruvate utilization, oxidative phosphorylation, ATP production, and cellular redox homeostasis. Experimental studies in insulin-responsive hepatic models have demonstrated that FOXK1/FOXK2 depletion alters transcriptional programs associated with lipid metabolism and mitochondrial function and produces measurable changes in mitochondrial respiration and fatty-acid oxidation [4]. These findings indicate that FOXK proteins contribute to the coordination of cellular metabolism with mitochondrial energy utilization rather than acting exclusively as regulators of glycolytic metabolism.

The relationship between FOXK proteins and mitochondria is further supported by independent evidence for FOXK2. El-Mergawy et al. demonstrated that FOXK2 can be detected in mitochondrial fractions and that depletion of FOXK2 in lung epithelial models impairs mitochondrial respiratory function [27]. The study additionally identified FBXO24-mediated ubiquitin-dependent degradation as a mechanism regulating FOXK2 abundance. These findings suggest that FOXK2 can influence mitochondrial energetics through mechanisms extending beyond its established nuclear transcriptional functions [27]. However, because this study was performed primarily in lung epithelial and experimental bacterial-infection models, the mitochondrial role of FOXK2 should not yet be generalized to all tissues or cancer types.

6.1. Mitochondrial Fatty-Acid Metabolism

Mitochondrial fatty-acid oxidation (FAO) provides acetyl-CoA, reducing equivalents, and ATP and therefore represents an important mechanism of metabolic adaptation. FOXK1 and FOXK2 participate in the transcriptional regulation of lipid-metabolic pathways and influence mitochondrial fatty-acid utilization. In insulin-responsive hepatic models, combined depletion of FoxK1 and FoxK2 altered lipid-metabolic gene expression and reduced mitochondrial fatty-acid oxidation, indicating that these factors contribute to the coordination of lipid utilization with cellular metabolic status [4].

More direct evidence for FOXK2-dependent lipid metabolic remodeling has recently emerged from cervical cancer. Liao et al. demonstrated that FOXK2 overexpression increased expression of carnitine palmitoyltransferase 1A (CPT1A) and enhanced FAO-associated metabolic activity, whereas FOXK2 depletion produced the opposite phenotype. In parallel, FOXK2 overexpression was associated with increased expression of acetyl-CoA carboxylase 1 (ACC1) and fatty acid synthase (FASN) in the context of mTOR modulation, demonstrating that FOXK2 participates in the regulation of both fatty-acid oxidation and lipogenic pathways [27].

The same study identified a functional relationship between FOXK2 and the mTOR/DRP1 signaling axis. FOXK2 interacted with mTOR and increased mTOR phosphorylation, while manipulation of FOXK2 altered DRP1 expression. Pharmacological inhibition or activation of the pathway partially modified the metabolic phenotype produced by FOXK2 manipulation [26]. These observations support a FOXK2–mTOR/DRP1 regulatory axis in cervical cancer, although the precise biochemical order of events and whether FOXK2 directly phosphorylates mTOR require further investigation.

Thus, FOXK2 may influence mitochondrial lipid utilization through both transcriptional and signaling mechanisms. Nevertheless, the FOXK2–mTOR/DRP1–FAO mechanism has currently been demonstrated most directly in cervical-cancer models and should not be extrapolated automatically to hepatic or other tumor contexts.

6.2. Oxidative Phosphorylation

Oxidative phosphorylation (OXPHOS) depends on the coordinated activity of the mitochondrial electron-transport chain and ATP synthase. FOXK1/FOXK2 depletion in insulin-responsive hepatic cellular models produces changes in mitochondrial respiration and fatty-acid oxidation, indicating that these transcription factors contribute to the cellular environment required for mitochondrial energy metabolism [4].

The 2019 study measured mitochondrial oxygen consumption using extracellular-flux analysis and demonstrated alterations

in basal respiration, ATP production, and maximal respiratory capacity following FOXK1/FOXK2 perturbation [4]. These findings provide functional evidence that FOXK proteins influence mitochondrial bioenergetics, although they do not establish that FOXK1 or FOXK2 directly regulates individual respiratory-chain complexes.

Independent evidence for FOXK2 was provided by El-Mergawy et al., who showed that FOXK2 depletion impaired mitochondrial respiratory function in lung epithelial cells [27]. FOXK2 was detected within mitochondrial fractions, suggesting that its influence on mitochondrial energetics may involve both nuclear transcriptional regulation and a potential mitochondrial component [27].

Importantly, FOXK1/FOXK2-dependent regulation of mitochondrial metabolism should not be interpreted as a simple increase or decrease in OXPHOS. The same transcription factors can promote aerobic glycolysis while influencing mitochondrial substrate utilization. Sukonina et al. demonstrated that FOXK1 and FOXK2 increase glycolytic enzyme expression and promote lactate production while simultaneously reducing mitochondrial pyruvate oxidation through regulation of pyruvate dehydrogenase kinase activity [11]. Therefore, FOXK-dependent metabolic regulation appears to support metabolic flexibility rather than constitutive activation of either glycolysis or OXPHOS.

6.3. Respiratory Capacity

Respiratory capacity reflects the ability of mitochondria to increase oxidative metabolism when cellular energy requirements rise. This parameter is particularly important for metabolically adaptable cells because spare respiratory capacity provides an energetic reserve that can be recruited during increased metabolic demand.

Experimental depletion of FOXK1 and FOXK2 in hepatic cellular models altered mitochondrial respiratory parameters, including basal respiration, ATP production, and maximal respiratory capacity [4]. These observations indicate that FOXK proteins contribute to maintaining mitochondrial bioenergetic competence.

FOXK2-specific evidence is also available from lung epithelial models. El-Mergawy et al. demonstrated that depletion of FOXK2 impaired mitochondrial respiration and reduced functional respiratory capacity [27]. These findings provide independent support for a role of FOXK2 in mitochondrial energetics, although the cellular context differs from the hepatic insulin-responsive models used by Sakaguchi et al. [4,27].

From a cancer perspective, changes in respiratory capacity may influence the ability of tumor cells to tolerate fluctuations in oxygen and nutrient availability. However, the current evidence supports FOXK proteins as regulators of mitochondrial metabolic fitness rather than establishing that FOXK1/FOXK2 universally increase respiratory capacity in cancer cells.

6.4. Mitochondrial Morphology and Dynamics

Mitochondrial metabolism is closely interconnected with mitochondrial morphology and dynamics. Fusion and fission continuously remodel the mitochondrial network in response to energetic and cellular demands. Dynamin-related protein 1 (DRP1), a GTPase involved in mitochondrial fission, is an important regulator of this process.

Evidence from insulin-responsive hepatic models indicates that FOXK1/FOXK2 depletion alters mitochondrial morphology and is accompanied by changes in mitochondrial metabolic function [4]. These observations suggest that FOXK-dependent transcriptional regulation may influence mitochondrial structure indirectly through changes in metabolic state and mitochondrial regulatory pathways.

More direct evidence has emerged for FOXK2 in cervical cancer. Liao et al. identified an interaction between FOXK2 and mTOR and demonstrated an association between the FOXK2–mTOR pathway and DRP1 expression [26]. Manipulation of FOXK2 altered mTOR phosphorylation and DRP1 abundance, while pharmacological manipulation of mTOR modified the metabolic phenotype associated with FOXK2 [26]. These findings provide experimental support for a FOXK2–mTOR/DRP1 signaling axis linking FOXK2 to mitochondrial dynamics and lipid metabolic remodeling in cervical-cancer cells.

Nevertheless, the current evidence should be described as an association between FOXK2, mTOR, DRP1, and mitochondrial metabolic remodeling rather than as proof that FOXK2 directly controls all aspects of mitochondrial fission. Additional studies measuring mitochondrial morphology, DRP1 recruitment to mitochondria, fission/fusion kinetics, and mitochondrial ultrastructure would help define the mechanism more precisely.

6.5. Nuclear–Mitochondrial Communication

Mitochondrial function depends on extensive communication between the nuclear and mitochondrial genomes. Most mitochondrial proteins are encoded by nuclear genes and subsequently imported into mitochondria. Consequently, transcription factors that regulate nuclear genes involved in mitochondrial metabolism can substantially influence mitochondrial bioenergetics.

FOXK1 and FOXK2 appear to participate in this nuclear–mitochondrial regulatory network. In hepatic models, depletion of FOXK1/FOXK2 produced broad transcriptional changes involving mitochondrial metabolism and was accompanied by functional alterations in mitochondrial fatty-acid oxidation and respiration [4]. These findings support a model in which FOXK-dependent nuclear transcription contributes to the maintenance of mitochondrial metabolic capacity.

FOXK2 may additionally influence mitochondrial function through its subcellular localization. El-Mergawy et al. detected FOXK2 in mitochondrial fractions and demonstrated that depletion of FOXK2 impaired mitochondrial function in lung epithelial cells [27]. These

observations raise the possibility that FOXK2 has mitochondrial functions that are not fully explained by its nuclear transcriptional activity. However, the molecular targets of mitochondrial FOXK2 and the functional significance of its mitochondrial localization remain incompletely defined [27].

Accordingly, it is more appropriate to describe FOXK1/FOXK2 as regulators of nuclear–mitochondrial metabolic coordination rather than established master regulators of mitochondrial biogenesis. Future studies using mitochondrial proteomics, isotope-tracing approaches, mitochondrial DNA analysis, respiratory-complex profiling, and real-time metabolic-flux measurements will be required to define the full extent of FOXK-dependent mitochondrial regulation.

6.5.1. Integrated Mechanistic Interpretation

The available experimental evidence supports a model in which insulin and nutrient-responsive signaling pathways regulate FOXK1/FOXK2 and thereby influence coordinated metabolic and mitochondrial adaptation:
Insulin/nutrients → IR/IRS → PI3K–AKT → mTORC1 and GSK3 regulation → FOXK1/FOXK2 → metabolic transcriptional remodeling → glycolysis + lipid metabolism + mitochondrial adaptation [4].

At the level of glucose metabolism:
FOXK1/FOXK2 → ↑ glycolytic enzymes → ↑ glycolytic flux → ↑ lactate production [11].
FOXK1/FOXK2 additionally increase PDK1/PDK4-associated regulation of the pyruvate dehydrogenase complex, resulting in increased inhibitory phosphorylation of PDH and reduced mitochondrial pyruvate oxidation, thereby favoring conversion of pyruvate to lactate [11].

For FOXK1, mTORC1 provides an additional regulatory mechanism. mTORC1 suppresses GSK3-dependent phosphorylation of FOXK1, thereby promoting FOXK1-dependent metabolic transcription and contributing to metabolic reprogramming [28].

In cervical cancer, a more tissue-specific mechanism has been reported:
FOXK2 → mTOR phosphorylation/interaction → DRP1 regulation → lipid-metabolic remodeling → altered CPT1A/FAO and ACC1/FASN programs → increased proliferation and invasion [26].

At the mitochondrial level, the evidence can therefore be summarized as:
FOXK1/FOXK2 → mitochondrial metabolic gene programs → fatty-acid utilization + respiratory adaptation
and, particularly in cervical-cancer models:
FOXK2 → mTOR/DRP1-associated signaling → mitochondrial dynamics/lipid metabolic remodeling [26].

Collectively, these observations support the concept that FOXK1 and FOXK2 function as metabolic transcriptional regulators linking nutrient- and insulin-responsive signaling to mitochondrial substrate utilization and cellular energy metabolism. However, their effects are strongly context dependent. The strongest evidence for insulin-dependent FOXK1/FOXK2 regulation comes from experimental hepatic models, whereas the FOXK2–mTOR/DRP1–lipid metabolism pathway is currently supported most directly in cervical-cancer models, and mitochondrial FOXK2 localization and respiratory regulation have been demonstrated in lung epithelial models [4,26,27]. This distinction is important when extrapolating these mechanisms to human metabolic disease or cancer as shown in Figure 2.

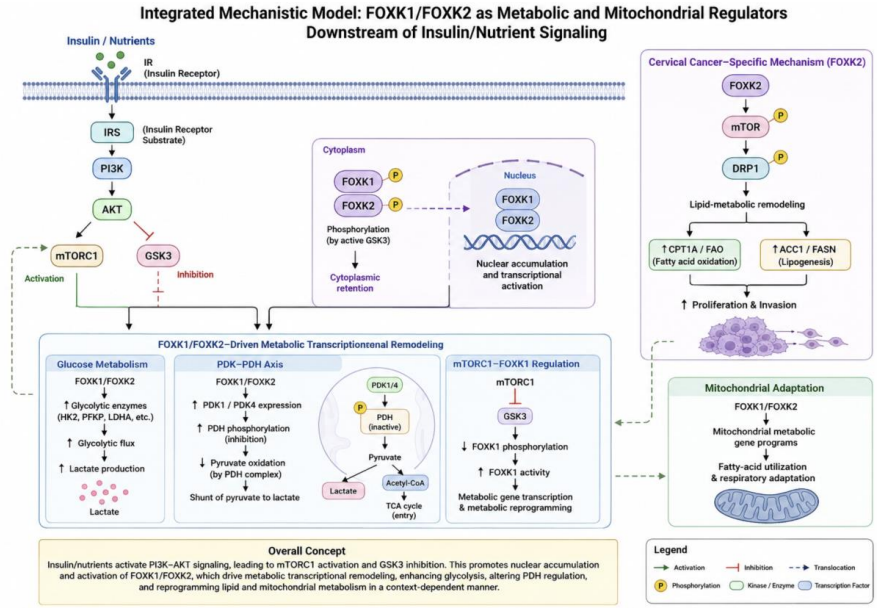

Figure 2: Integrated Mechanistic Model FOXK1/FOXK2 as Metabolic and Mitochondrial Regulators Downstream of Insulin/Nutrient signalling

7. FOXK1/FOXK2 in Hepatic Insulin Action

The liver is a major site of systemic glucose and lipid homeostasis and is highly responsive to changes in insulin availability. Insulin binding to the insulin receptor (IR) activates IRS proteins and downstream PI3K–AKT signaling, which coordinates hepatic glucose production, lipid metabolism, protein synthesis, and cellular growth. Although FOXO1 has traditionally been regarded as the principal forkhead transcription factor mediating insulin-dependent regulation of hepatic gluconeogenesis, experimental studies have identified FOXK1 and FOXK2 as additional insulin-responsive transcriptional regulators [4,9,10].

Sakaguchi et al. demonstrated that insulin stimulation promotes redistribution of FOXK1 from the cytoplasm toward the nucleus and chromatin, in a manner dependent on PI3K–AKT–mTOR signaling, whereas GSK3 activity contributes to basal cytoplasmic retention [4]. Similar insulin-responsive behavior was observed for FOXK2, although FOXK2 displayed a greater basal nuclear distribution than FOXK1 [4]. These findings establish FOXK1/FOXK2 as components of insulin-responsive transcriptional regulation, but the strongest mechanistic evidence currently concerns FOXK1 and experimental hepatic models.

7.1. FOXK1 in Hepatic Transcriptional Regulation

FOXK1 participates in the regulation of hepatic metabolic gene expression and responds dynamically to insulin-associated signaling. Under basal conditions, GSK3-dependent phosphorylation contributes to cytoplasmic retention of FOXK1. Insulin activates PI3K–AKT signaling and promotes mTOR-dependent nuclear accumulation of FOXK1, thereby increasing its access to chromatin [4].

Importantly, the role of FOXK1 in hepatic glucose metabolism is supported by direct functional experiments. Wang et al. used DNA-affinity purification followed by mass spectrometry to identify insulin-responsive transcription factors associated with the promoters of Pck1 and G6pc, two major genes involved in hepatic glucose production [10]. FOXK1 was among the insulin-responsive factors identified, and its binding to the Pck1 insulin-response region required an intact insulin-response sequence. Knockdown of FoxK1 in primary hepatocytes reduced glucose production and impaired the ability of insulin to suppress hepatic glucose output [10]. These findings provide direct evidence that FOXK1 contributes to insulin-dependent regulation of hepatic glucose production.

Genome-wide analysis has subsequently expanded this model. Allu et al. demonstrated that FOXK1 occupies more than 4000 proximal promoters and enhancers in hepatic chromatin and that insulin increases FOXK1 association with approximately 75% of these sites [9]. FOXK1-associated genes included pathways involved in glucose and lipid metabolism, mitochondrial function, cell-cycle regulation, steroid biosynthesis, autophagy, and cellular senescence [9].

The same study identified the forkhead consensus motif TGTTTAC as a major FOXK1-associated DNA-binding sequence and suggested that hepatocyte nuclear factor 4α (HNF4α) may participate in FOXK1-associated transcriptional regulation [9]. FOXK1 also occupied regulatory regions associated with genes such as PCK1, G6PC, PKM, LARS1, and TIMM22, illustrating that its hepatic regulatory function extends beyond classical glucose metabolism [9].

These observations support the interpretation that FOXK1 functions as a broad hepatic transcriptional regulator rather than as a single-gene regulator of gluconeogenesis. Its activity integrates insulin-dependent signaling with transcriptional programs governing glucose metabolism, lipid metabolism, mitochondrial function, and cellular growth [4,9,10].

7.2. Interaction with Insulin-Receptor-Associated Signaling

The regulation of FOXK1/FOXK2 by insulin involves the PI3K–AKT–mTOR and GSK3 signaling network. Insulin receptor activation leads to IRS-dependent PI3K activation and subsequent AKT signaling. Experimental inhibition of PI3K or AKT prevents insulin-induced nuclear accumulation of FOXK1, whereas inhibition of MEK/ERK has comparatively limited effects [4]. These observations indicate that the PI3K–AKT branch is particularly important for insulin-dependent FOXK1 redistribution.

The role of GSK3 is complementary. In the basal state, GSK3 activity promotes FOXK1/FOXK2 cytoplasmic retention. Pharmacological inhibition or depletion of GSK3α/β promotes nuclear accumulation of both FOXK1 and FOXK2, even in the absence of insulin [4]. In addition, mTOR inhibition prevents insulin-induced FOXK1 nuclear localization, indicating that mTOR signaling contributes to the regulation of FOXK nuclear activity [4].

The resulting regulatory framework can therefore be represented as:
Insulin → IR → IRS → PI3K → AKT → mTOR signaling + reduced GSK3 restraint → FOXK1/FOXK2 nuclear accumulation → chromatin association → metabolic gene regulation [4].

This mechanism provides a molecular link between insulin receptor signaling and transcriptional regulation. Importantly, FOXK proteins are not simply passive downstream targets. Sakaguchi et al. demonstrated that depletion of either FOXK1 or FOXK2 increased insulin-stimulated phosphorylation of AKT, ERK1/2, and ribosomal S6 protein without substantially changing IR or IRS1 phosphorylation [4]. Thus, FOXK proteins can also modulate the magnitude of intracellular insulin signaling, suggesting the existence of transcriptionally mediated feedback.

7.3. FOXK1 and FOXO1 Regulatory Relationships

FOXO1 remains a central transcriptional regulator of hepatic glucose production. During fasting or reduced insulin signaling, FOXO1 accumulates in the nucleus and promotes expression of

gluconeogenic genes, including PCK1 and GPC. Insulin activates AKT, which phosphorylates FOXO1 and promotes its redistribution from the nucleus to the cytoplasm, thereby suppressing FOXO1-dependent transcription [4,18].

FOXK1 displays a strikingly reciprocal response. Insulin promotes FOXK1 nuclear accumulation while promoting FOXO1 nuclear exclusion [4]. This reciprocal regulation provides a potential mechanism by which insulin can simultaneously suppress fasting-associated transcription and activate or reinforce alternative metabolic programs.

Genome-wide hepatic analysis provides stronger evidence for functional crosstalk between FOXK1 and FOXO1. Allu et al. found that FOXK1 and FOXO1 occupy overlapping subsets of insulin-responsive regulatory regions and participate in partially overlapping gene-regulatory programs [9]. FOXK1 was also associated with genes involved in mitochondrial translation, amino-acid metabolism, cellular senescence, and mitochondrial biogenesis-related pathways [9].

However, these observations should not be interpreted as evidence that FOXK1 and FOXO1 invariably interact physically. The current evidence more strongly supports functional and genomic crosstalk, involving overlapping chromatin occupancy and complementary responses to insulin signaling [9].

Thus, FOXK1 and FOXO1 should be considered components of an interconnected hepatic insulin-responsive transcriptional network. FOXO1 is particularly important for fasting-associated gluconeogenic transcription, whereas FOXK1 contributes to broader insulin-responsive programs involving glucose metabolism, lipid metabolism, mitochondrial function, and cellular growth [9,10].

7.4. Human Hepatic Evidence

Although experimental evidence supporting FOXK1/FOXK2 involvement in hepatic insulin action is substantial, direct evidence from human liver remains limited. Much of the mechanistic literature has been generated using cultured hepatocyte models, mouse-derived hepatic cells, genetically manipulated animals, and primary hepatocytes [4,10].

Sakaguchi et al. demonstrated insulin-dependent FOXK1 nuclear redistribution in AML12 liver cells and also observed FOXK1 redistribution in mouse liver following insulin administration [4]. Wang et al. demonstrated a functional contribution of FOXK1 to insulin-regulated glucose production in primary hepatocytes [10]. These findings provide strong experimental support for FOXK1 as an insulin-responsive hepatic transcription factor, but they do not establish the quantitative importance of FOXK1/FOXK2 in normal or insulin-resistant human liver.

The 2023 genome-wide study by Allu et al. substantially strengthened the hepatic evidence by demonstrating extensive FOXK1 chromatin occupancy and insulin-dependent changes in its association with hepatic regulatory regions [9]. Nevertheless, the study does not eliminate the need for human translational studies.

This limitation is particularly relevant to metabolic disease. Human insulin resistance is associated with chronic alterations in insulin concentrations, glucose availability, lipid accumulation, inflammation, and mitochondrial metabolism. These conditions may alter FOXK1/FOXK2 expression, localization, phosphorylation, chromatin occupancy, or cofactor availability. Therefore, the behavior of FOXK proteins under chronic insulin-resistant conditions cannot be inferred solely from acute insulin-stimulation experiments.

Future investigations using human liver biopsies, primary human hepatocytes, human liver organoids, single-cell and single-nucleus transcriptomics, chromatin-accessibility profiling, ChIP-seq/CUT&RUN, and metabolic-flux analysis will be important for determining the clinical relevance of FOXK-dependent hepatic transcription.

7.5. Limitations of Current Experimental Models

Several limitations should be considered when interpreting the current hepatic FOXK literature.

First, a substantial proportion of mechanistic studies have used immortalized or transformed hepatocyte-derived cell systems. Such models provide important mechanistic advantages but may not fully reproduce the metabolic characteristics of primary human hepatocytes or intact human liver.

Second, animal models provide valuable evidence for insulin-dependent FOXK regulation in vivo, but species-specific differences in hepatic glucose and lipid metabolism may limit direct extrapolation to humans [4].

Third, FOXK1 and FOXK2 have frequently been studied together. Consequently, some phenotypes may reflect overlapping or compensatory functions rather than identical biological activities. Sakaguchi et al. showed that combined FOXK1/FOXK2 depletion produced particularly broad transcriptional and metabolic effects, whereas individual depletion produced more restricted phenotypes [4].

Fourth, FOXK expression alone does not necessarily represent FOXK transcriptional activity. Nuclear localization, phosphorylation, protein stability, DNA occupancy, and interactions with transcriptional cofactors can all influence the functional state of FOXK proteins [4,9].

Fifth, acute insulin stimulation experiments do not fully reproduce chronic metabolic disease. Obesity, type 2 diabetes, hyperinsulinemia, hyperglycemia, ectopic lipid accumulation, inflammation, and mitochondrial dysfunction develop over prolonged periods and may generate regulatory states that differ substantially from those observed following short-term insulin

exposure.

Finally, although FOXK1 has strong experimental support as a hepatic insulin-responsive transcriptional regulator, the specific contribution of FOXK2 to human hepatic insulin action remains less well defined. Therefore, statements regarding FOXK2 should generally be framed as evidence-supported but less extensively characterized rather than equivalent to the evidence available for FOXK1.

Overall, current evidence supports a model in which FOXK1, and to a lesser extent FOXK2, contributes to the transcriptional response of hepatocytes to insulin. FOXK1 is particularly well supported as a regulator of hepatic glucose production and insulin-responsive chromatin, while FOXK1/FOXK2 collectively influence broader metabolic, mitochondrial, proliferative, and survival programs. Their reciprocal regulation with FOXO1 further expands the classical insulin-responsive transcriptional network and provides a framework for understanding how insulin coordinates suppression of fasting metabolism with activation of nutrient-responsive cellular programs [4,9,10].

8. FOXK1/FOXK2 in Cellular Proliferation and Survival

Beyond metabolic regulation, FOXK1 and FOXK2 participate in cellular proliferation, survival, differentiation, and stress responses. Their metabolic functions are closely connected to these processes because proliferating cells require coordinated production of ATP and biosynthetic intermediates. FOXK proteins therefore provide a mechanistic bridge between nutrient sensing, metabolic reprogramming, and cell-cycle progression [4,17].

8.1. G1/S Transition

Progression from G1 into S phase requires increased biosynthetic capacity and coordinated activation of DNA replication machinery. FOXK1 has been implicated in transcriptional programs supporting cell-cycle progression, particularly through regulation of genes associated with proliferation and DNA synthesis [22].

FOXK1 can promote expression of cell-cycle-associated genes and contribute to the transition from a metabolically quiescent state toward active proliferation. This function is particularly relevant in cancer, where persistent FOXK1 activity can couple metabolic sufficiency with cell-cycle progression.

FOXK2 also contributes to proliferative programs in several cancer models. However, the exact mechanisms and downstream targets vary according to cellular context. Therefore, FOXK1/FOXK2 should be considered context-dependent regulators of G1/S progression rather than universal drivers of cell-cycle entry.

8.2. Cell-Cycle Gene Regulation

FOXK proteins regulate transcriptional networks involved in proliferation, including genes associated with DNA replication, chromatin regulation, and cell-cycle control. FOXK1 has been linked to transcriptional regulation of genes required for cellular proliferation and maintenance of proliferative capacity [22].

FOXK2 also interacts with chromatin-associated regulatory proteins and can influence transcriptional complexes involved in cell growth and DNA-damage responses. Its biological activity therefore extends beyond metabolic regulation to the control of transcriptional states that determine whether cells proliferate, differentiate, or respond to stress [21,24].

The integration of metabolic and cell-cycle regulation is particularly important because insufficient metabolic resources can activate cell-cycle checkpoints, whereas enhanced nutrient utilization can support sustained proliferation. FOXK proteins may therefore function at the interface between these two biological systems.

8.3. Apoptotic Signaling

FOXK1/FOXK2 can influence cell survival by regulating transcriptional pathways associated with stress responses and apoptosis. FOXK2 has been implicated in cellular responses to DNA damage and transcriptional control of genes involved in survival and stress adaptation [21,24].

The relationship between FOXK proteins and apoptosis is context dependent. In some settings, FOXK2-associated transcriptional complexes support cellular survival, whereas under particular stress conditions FOXK2 can participate in transcriptional programs that facilitate checkpoint activation. Consequently, FOXK2 should not be classified exclusively as either an anti-apoptotic or pro-apoptotic factor.

In cancer, increased FOXK activity may favor survival by simultaneously supporting metabolic adaptation, DNA-damage responses, and proliferative transcription. This combination could contribute to resistance to environmental and therapeutic stress.

8.4. Nutrient Availability and Cell Fate

Cellular nutrient availability strongly influences whether cells proliferate, remain quiescent, differentiate, or undergo apoptosis. FOXK1/FOXK2 are well positioned to participate in this decision because their activity is regulated by insulin- and mTOR-associated nutrient signaling [4,17].

When nutrients and growth factors are abundant, FOXK activation can promote glycolysis, lipid utilization, and biosynthetic pathways that support proliferation. Conversely, reduced nutrient signaling may alter FOXK phosphorylation, localization, and transcriptional activity. FOXK proteins therefore provide a mechanism through which metabolic status can influence transcriptional decisions concerning cellular growth and survival.

This model may be particularly relevant to tumors, in which nutrient availability is heterogeneous. Cancer cells expressing elevated FOXK proteins may be better able to switch between metabolic pathways and maintain proliferation during metabolic stress.

9. FOXK1/FOXK2 in Metabolic and Human Disease

The involvement of FOXK1 and FOXK2 in insulin-responsive

signaling, glucose metabolism, lipid utilization, mitochondrial function, and cellular proliferation provides a plausible mechanistic basis for their contribution to metabolic and human disease. However, the strength of evidence differs substantially among disease settings. Experimental studies provide convincing mechanistic evidence for FOXK-dependent regulation of cellular metabolism, whereas direct evidence establishing FOXK1/FOXK2 as causal determinants of human metabolic disease remains limited [4,11,17,29].

9.1. Insulin Resistance

Insulin resistance is characterized by impaired cellular responses to insulin and disruption of glucose, lipid, and energy homeostasis. FOXK1 and FOXK2 are regulated by insulin through the AKT–mTOR and GSK3 signaling pathways and undergo insulin-dependent changes in subcellular localization. In experimental hepatic models, depletion of FoxK1 or FoxK2 alters the expression of genes involved in metabolism, mitochondrial function, proliferation, and apoptosis [4]. These findings establish FOXK1/FOXK2 as insulin-responsive transcriptional regulators but do not, by themselves, demonstrate that FOXK dysregulation is a primary cause of insulin resistance.

Chronic nutrient excess and impaired insulin signaling could potentially modify FOXK phosphorylation, localization, protein stability, or transcriptional activity. Such changes may subsequently influence glycolytic metabolism, lipid utilization, and mitochondrial function. However, a direct causal relationship between altered FOXK activity and systemic insulin resistance has not yet been established in humans. Therefore, FOXK1/FOXK2 are better regarded at present as potential components of the molecular response to altered nutrient and insulin signaling rather than established primary drivers of insulin resistance [4,29].

Future studies should determine whether FOXK1/FOXK2 activity is altered during the development of insulin resistance and whether manipulation of these factors can prevent or reverse impaired insulin action in physiologically relevant models.

9.2. Obesity and Metabolic Dysfunction

Obesity is associated with chronic nutrient excess, adipose-tissue dysfunction, inflammation, altered lipid handling, and impaired insulin sensitivity. FOXK1 and FOXK2 regulate several metabolic processes affected by nutrient availability, including aerobic glycolysis, lipid metabolism, and mitochondrial metabolism [11,17]. Experimental studies therefore provide a mechanistic basis for investigating whether dysregulated FOXK activity contributes to metabolic adaptation during obesity.

Nevertheless, the current evidence does not justify identifying FOXK1 or FOXK2 as established causal determinants of human obesity. The available literature predominantly derives from cellular and animal models, and the relationship between FOXK activity, adiposity, systemic insulin sensitivity, and whole-body energy expenditure remains incompletely defined [11,17].

An important consideration is that FOXK-dependent metabolic responses may be tissue specific. Effects observed in hepatocytes, adipocytes, skeletal muscle, or cancer cells cannot necessarily be extrapolated to whole-body metabolic physiology. Consequently, future studies should examine FOXK1/FOXK2 expression and activity in metabolically relevant tissues in relation to insulin sensitivity, hepatic steatosis, adiposity, and metabolic biomarkers.

9.3. Liver Disease

The liver represents one of the most important physiological contexts for FOXK1 biology because FOXK proteins regulate hepatic lipid metabolism and respond to nutrient-sensitive signaling [4,17]. This relationship is particularly relevant to metabolic dysfunction-associated steatotic liver disease (MASLD), in which excessive lipid accumulation, altered fatty-acid metabolism, mitochondrial dysfunction, inflammation, and fibrosis contribute to disease progression.

Importantly, experimental evidence now directly implicates FOXK1 in fatty-liver disease. Hepatocyte-specific Foxk1 deficiency was shown to ameliorate diet-induced hepatic steatosis, inflammation, fibrosis, and associated hepatocellular carcinoma development in mice. Mechanistically, FOXK1 promoted nutrient-dependent suppression of hepatic fatty-acid oxidation through an mTORC1-dependent pathway, and Ppara was identified among lipid-metabolism-related FOXK1 target genes [30]. These findings provide substantially stronger evidence for a role of FOXK1 in hepatic metabolic disease than would be suggested by purely associative studies.

However, these findings should not be interpreted as proof that FOXK1 drives MASLD in humans. The experimental evidence is predominantly derived from mouse models and mechanistic studies, and the extent to which the FOXK1–mTORC1–fatty-acid oxidation pathway operates similarly in human MASLD remains to be determined. FOXK2 may also participate in hepatic metabolic regulation, but its specific contribution to MASLD requires further investigation [4,29].

Thus, FOXK1 represents a potentially important regulator of hepatic lipid homeostasis and a candidate pathway linking nutrient sensing with steatotic liver disease, whereas FOXK2 remains less well characterized in this specific disease context.

9.4. Cancer and Metabolic Reprogramming

Among disease settings, cancer currently provides some of the strongest evidence linking FOXK proteins to metabolic reprogramming. FOXK1 and FOXK2 can regulate glycolytic metabolism by increasing the expression of glycolytic enzymes and promoting conversion of glucose-derived pyruvate to lactate [11]. They can also influence mitochondrial metabolism and cellular responses to nutrient availability, thereby providing proliferating cells with metabolic flexibility [4,11].

FOXK1 has additionally been linked to mTORC1-dependent metabolic reprogramming. mTORC1 suppresses GSK3-dependent

FOXK1 phosphorylation, thereby promoting FOXK1 activity and contributing to transcriptional programs associated with glycolytic and anabolic metabolism [17]. These findings provide a mechanistic connection between nutrient-sensitive signaling and persistent metabolic gene regulation.

More recent evidence has directly connected FOXK2 with lipid metabolic reprogramming in cervical cancer. Liao et al. demonstrated that FOXK2 overexpression increased CPT1A expression and fatty-acid oxidation while reducing expression of the lipogenic enzymes ACC1 and FASN. FOXK2 also increased mTOR phosphorylation and interacted with mTOR, while mTOR interacted with DRP1. Genetic and pharmacological experiments supported involvement of the FOXK2–mTOR/DRP1 axis in lipid metabolic remodeling and cervical cancer progression [26].

The experimental model can therefore be summarized as:
FOXK2 → mTOR activation/interaction → DRP1 regulation → lipid metabolic remodeling → increased fatty-acid utilization → enhanced cervical cancer proliferation and invasion [26].

This mechanism is particularly relevant because it demonstrates that FOXK2 can connect transcriptional regulation with signaling-dependent control of lipid metabolism and tumor progression. However, the evidence currently derives primarily from cervical cancer cell models and xenograft experiments. Therefore, this pathway should be described as an experimentally supported mechanism in cervical cancer rather than a universal mechanism operating across all malignancies.

An additional consideration is that FOXK2 may have either tumor-promoting or tumor-suppressive functions depending on cellular and tissue context. Recent reviews summarize evidence for apparently opposing FOXK2 functions across different cancers [20,29]. Consequently, increased FOXK2 activity should not be universally interpreted as oncogenic.

9.5. Tissue- and Context-Specific Functions

A central feature of FOXK biology is its context dependence. FOXK1 and FOXK2 can promote aerobic glycolysis while also influencing mitochondrial metabolism and fatty-acid utilization [11,17]. This apparent coexistence of glycolytic and oxidative metabolic programs should not necessarily be interpreted as contradictory. Rather, FOXK proteins may contribute to metabolic flexibility by coordinating the relative use of different carbon and energy sources according to cellular state.

The balance between these effects is likely determined by tissue type, nutrient availability, insulin/mTOR signaling, differentiation state, chromatin environment, and the availability of transcriptional cofactors [4,11,17,29]. Consequently, FOXK1/FOXK2 should not be classified simply as either “glycolytic” or “mitochondrial” transcription factors. A more appropriate interpretation is that they function as context-dependent regulators of metabolic adaptation.

This distinction has important implications for therapeutic development. Systemic inhibition of FOXK activity could potentially interfere with physiological metabolic functions in normal tissues while producing antitumor effects in cancers that are dependent on FOXK-mediated transcriptional programs. Moreover, because FOXK1 and FOXK2 are not completely redundant, selective targeting of one factor may produce different biological consequences from simultaneous inhibition of both proteins [4,29].

Overall, current evidence supports FOXK1/FOXK2 as emerging regulators at the intersection of nutrient signaling, metabolic adaptation, mitochondrial function, and disease progression. The strongest mechanistic evidence currently concerns experimental models of metabolic regulation and cancer, including FOXK1-dependent hepatic lipid metabolism and FOXK2-dependent lipid metabolic remodeling in cervical cancer [4,26,30]. Definitive studies in human tissues are nevertheless required to establish whether these mechanisms represent clinically relevant determinants of insulin resistance, obesity, MASLD, or cancer metabolism.

10. Therapeutic Implications

The involvement of FOXK1 and FOXK2 in insulin-responsive signaling, metabolic reprogramming, mitochondrial function, and cellular proliferation provides a rationale for investigating these transcription factors as potential therapeutic vulnerabilities. Experimental studies have established that FOXK1 and FOXK2 respond to insulin-associated AKT–mTOR/GSK3 signalling and regulate metabolic and cellular programs downstream of nutrient sensing [4,11]. However, direct pharmacological targeting of FOXK1 or FOXK2 remains an emerging area of research, and no FOXK1- or FOXK2-selective therapeutic inhibitor has yet been established for clinical use. Therefore, the current therapeutic relevance of FOXK proteins is better considered in terms of metabolic vulnerabilities, pathway dependencies, and regulatory interactions rather than as validated standalone drug targets.

10.1. FOXK1/FOXK2 as Potential Metabolic Vulnerabilities

FOXK1 and FOXK2 regulate multiple metabolic processes, including aerobic glycolysis, lipid metabolism, and mitochondrial function [11,17]. Their ability to coordinate several metabolic pathways raises the possibility that FOXK-dependent transcription may contribute to the metabolic adaptability required for sustained proliferation.

This concept is particularly relevant in cancer, where metabolic plasticity allows tumor cells to adapt to changes in glucose, oxygen, and nutrient availability. Experimental suppression of FOXK1 or FOXK2 alters glycolytic metabolism, while depletion of these factors in hepatic models affects lipid metabolism and mitochondrial function [4,11]. Thus, FOXK-dependent metabolic programs may represent potential vulnerabilities in tumors that become strongly dependent on these transcriptional networks.

However, direct inhibition of FOXK proteins could also interfere with physiological metabolic functions. FOXK1 and FOXK2 participate in normal cellular metabolism, proliferation, and mitochondrial regulation, and therefore systemic inhibition could potentially produce unwanted effects [4,11]. At present, this possibility remains a theoretical concern rather than a clinically demonstrated toxicity.

A more realistic therapeutic strategy may therefore be to identify tumor-specific FOXK dependencies and target the metabolic pathways that become essential downstream of FOXK activation rather than attempting indiscriminate systemic FOXK inhibition.

For example, the recent cervical-cancer study by Liao et al. demonstrated that FOXK2 promotes fatty-acid oxidation, increases CPT1A expression, suppresses ACC1 and FASN, and activates an mTOR/DRP1-associated signaling program [26]. These findings raise the possibility that tumors with a strong FOXK2-dependent fatty-acid metabolic phenotype could be evaluated for sensitivity to interventions targeting fatty-acid oxidation or associated signaling pathways. Nevertheless, this remains a preclinical hypothesis requiring validation.

10.2. Targeting Upstream AKT–mTOR–GSK3 Signalling

Because FOXK1/FOXK2 activity is regulated by nutrient-sensitive signalling pathways, modulation of upstream AKT, mTOR, or GSK3 signalling represents an indirect means of altering FOXK-dependent transcription.

mTORC1 is particularly relevant to FOXK1. He et al. demonstrated that suppression of mTORC1 increases GSK3-dependent phosphorylation of FOXK1, promotes 14-3-3 association, reduces FOXK1 DNA binding, and facilitates nuclear exclusion. FOXK1 subsequently contributes to mTORC1-associated glycolytic and anabolic metabolic reprogramming through direct transcriptional regulation and HIF-1α-associated mechanisms [17]. These findings establish a mechanistic connection between mTORC1 signalling and FOXK1-dependent metabolic gene expression.

Nevertheless, pharmacological inhibition of mTOR cannot be considered equivalent to FOXK1 inhibition. mTORC1 regulates a large network of metabolic, translational, autophagic, and growth-associated processes independently of FOXK1. Therefore, the biological effects of rapamycin or other mTOR-directed agents cannot be attributed specifically to disruption of FOXK1 activity.

Similarly, AKT inhibition may alter FOXK localisation and activity but simultaneously affects FOXO transcription factors, glucose metabolism, protein synthesis, apoptosis, and numerous other cellular processes [4]. Consequently, AKT or mTOR inhibitors should be regarded as pathway-level interventions that may modulate FOXK-dependent programs, rather than FOXK-specific therapeutic agents.

An important therapeutic implication is that FOXK1/FOXK2 status could potentially help identify tumours in which modulation of insulin–PI3K–AKT–mTOR signalling produces a particularly strong metabolic response. This possibility requires prospective experimental validation.

10.3. Challenges in Direct FOXK Targeting

Direct pharmacological targeting of FOXK1 and FOXK2 presents several challenges. As transcription factors, FOXK proteins primarily exert their functions through DNA binding, protein–protein interactions, and recruitment of chromatin-regulatory complexes rather than through a conventional enzymatic catalytic activity. Consequently, identifying selective small molecules that disrupt their function without affecting related FOX proteins may be difficult.

A second challenge is the complexity of FOXK-associated protein interactions. FOXK1 and FOXK2 interact with chromatin-associated cofactors and regulatory complexes, including SIN3-associated machinery and BAP1-containing complexes [14,31]. Recent work demonstrated that FOXK1 O-GlcNAcylation enhances BAP1 recruitment to E2F-regulated chromatin and promotes transcription of E2F target genes. Disruption of FOXK1 O-GlcNAcylation impaired BAP1 recruitment and reduced proliferation and tumour growth in experimental models [14]. This finding suggests that regulatory interfaces surrounding FOXK proteins may represent alternative therapeutic entry points.

A third challenge is functional overlap between FOXK1 and FOXK2. Because the two factors can compensate for one another at some genomic and metabolic targets, inhibition of a single factor may not completely suppress FOXK-dependent transcription [4]. Conversely, simultaneous inhibition could produce greater effects on normal tissues.

A fourth challenge is biomarker development. FOXK1 or FOXK2 expression alone may not accurately indicate functional pathway dependence. FOXK activity can also be influenced by phosphorylation, sub-cellular localisation, post-translational modification, protein stability, chromatin accessibility, and the availability of interacting cofactors [4,14,17]. Therefore, future therapeutic studies should incorporate functional biomarkers rather than relying exclusively on FOXK expression.

10.4. Opportunities for Precision Metabolic Therapy

The current evidence supports a precision-medicine approach in which FOXK-associated metabolic dependencies are identified before therapeutic intervention. Rather than treating FOXK expression as a universal therapeutic biomarker, tumours could be characterised according to FOXK1/FOXK2 abundance, transcriptional activity, metabolic phenotype, mTOR signalling, mitochondrial function, and dependence on glycolysis or fatty-acid oxidation.

A potential experimental framework is:
FOXK-high or FOXK-activated tumour → functional metabolic profiling → identification of FOXK-dependent metabolic pathway → pathway-selective intervention → assessment of therapeutic

response and resistance.

In FOXK2-high cervical cancer models with increased fatty-acid oxidation, for example, the FOXK2–mTOR/DRP1-associated metabolic pathway could be investigated using genetic and pharmacological approaches targeting fatty-acid oxidation or mTOR-associated signalling .76 Importantly, this should currently be described as a preclinical therapeutic hypothesis, rather than a clinically established treatment strategy.

Similarly, tumors displaying strong FOXK1/FOXK2-dependent glycolytic activity could be investigated for combinations involving metabolic pathway inhibition and established anticancer therapies. The rationale is supported by experimental evidence that FOXK1 and FOXK2 regulate aerobic glycolysis and lactate production [11]. However, metabolic inhibition alone may be insufficient because tumour cells can compensate by increasing alternative nutrient pathways.

The recent identification of FOXK1 O-GlcNAcylation as a regulator of BAP1 recruitment and E2F transcription also suggests another potential strategy: targeting FOXK1 regulatory modifications or protein–protein interactions rather than the FOXK1 DNA-binding domain itself [14]. Such approaches remain experimental but may ultimately provide greater selectivity than broad inhibition of FOXK transcriptional activity.

Before clinical translation, these concepts require validation in physiologically relevant models, including patient-derived organoids, genetically engineered tumor models, patient-derived xenografts, metabolic flux experiments, and eventually prospective biomarker-driven clinical studies.

10.5. Therapeutic Perspective and Future Directions

The therapeutic significance of FOXK1 and FOXK2 currently lies less in their immediate suitability as standalone drug targets and more in their potential to identify metabolic and transcriptional dependencies within specific disease contexts.

Several priorities should be addressed. First, selective FOXK1- and FOXK2-directed inhibitors or degraders need to be developed and experimentally validated. Second, functional biomarkers capable of distinguishing FOXK expression from FOXK transcriptional activity should be established. Third, tissue-specific consequences of FOXK inhibition need to be characterised to determine the therapeutic window. Fourth, the degree of functional compensation between FOXK1 and FOXK2 should be defined in individual tumor types. Finally, combination strategies should be evaluated to determine whether FOXK-dependent metabolic vulnerabilities can be exploited without producing unacceptable systemic metabolic toxicity.

Overall, current evidence supports FOXK1 and FOXK2 as emerging transcriptional nodes connecting insulin/nutrient signalling with metabolic adaptation and tumour biology [4,11,17]. Their therapeutic potential is promising but remains preclinical. The most defensible strategy at present is therefore not generalised FOXK inhibition but identification of context-specific FOXK dependencies and downstream metabolic vulnerabilities, particularly those involving glycolysis, fatty-acid oxidation, mTOR signalling, mitochondrial function, and FOXK-associated chromatin regulation.

11. Knowledge Gaps and Future Directions

Despite substantial progress in defining FOXK1 and FOXK2 as nutrient-responsive transcriptional regulators, important mechanistic and translational questions remain unresolved. Experimental studies demonstrate that insulin promotes redistribution of FOXK1 and FOXK2 from the cytoplasm toward the nucleus through an AKT–mTOR-dependent mechanism, whereas GSK3 activity contributes to their cytoplasmic localisation under basal conditions. FOXK1 and FOXK2 subsequently influence transcriptional programs involved in glucose metabolism, aerobic glycolysis, lipid metabolism, mitochondrial function, and cellular proliferation [4,11]. However, the relative contribution of each factor, the hierarchy connecting signaling-dependent post-translational modifications with transcriptional activity, and the extent to which these mechanisms operate in human disease remain incompletely defined [4,9,11].

Future research should therefore move beyond measurements of FOXK expression alone and integrate transcription-factor occupancy, chromatin accessibility, post-translational modification, transcriptional activity, metabolic flux, mitochondrial function, and physiological phenotypes. Such multidimensional approaches will be particularly important for distinguishing primary FOXK-dependent mechanisms from secondary consequences of altered cellular metabolism.

11.1. Direct Versus Indirect FOXK Targets

An important unresolved question in FOXK biology is the distinction between genes directly regulated by FOXK proteins and genes whose expression changes indirectly after FOXK perturbation. Transcriptomic and proteomic studies have identified broad changes in metabolic, mitochondrial, and cellular pathways following FOXK1/FOXK2 manipulation [4,11]. Nevertheless, differential expression alone cannot establish direct transcriptional regulation.

Strong evidence for direct FOXK1-dependent regulation in the liver was provided by Allu et al., who performed genome-wide FOXK1 chromatin immunoprecipitation sequencing (ChIP-seq) in hepatic models. FOXK1 occupied proximal promoters and enhancers associated with more than 4,000 genes, and insulin increased FOXK1 occupancy at approximately 75% of these sites. The associated genes included regulators of metabolism, mitochondrial function, cell-cycle progression, steroid biosynthesis, autophagy, and cellular senescence. Comparison with insulin receptor and FOXO1 chromatin datasets further suggested that FOXK1 participates in hepatic insulin-responsive gene regulation and may contribute to transcriptional effects previously attributed to insulin receptor signalling [9].

However, chromatin occupancy should not automatically be interpreted as transcriptional activation or repression. Binding of FOXK1 to a regulatory region may indicate a functional transcriptional site, a permissive chromatin environment, or a site whose activity depends on additional transcriptional cofactors. Future studies should therefore combine ChIP-seq, CUT&RUN, or CUT&Tag with nascent RNA sequencing, chromatin-accessibility profiling, and targeted gain- and loss-of-function experiments. These approaches would provide stronger evidence for distinguishing directly regulated FOXK targets from secondary transcriptional responses.

This distinction is particularly important for FOXK2 because genome-wide evidence defining its hepatic insulin-responsive regulatory network remains less extensive than that available for FOXK1 [4,9]. Comparative FOXK1 and FOXK2 chromatin profiling in the same experimental system, under basal, insulin-stimulated, nutrient-deprived, and nutrient-replete conditions, would help determine the degree of functional redundancy between the two factors.

Post-translational regulation represents another important unresolved layer. FOXK1 and FOXK2 are regulated by signalling-dependent phosphorylation, whereas recent work has provided direct evidence that FOXK1 is also regulated by O-GlcNAcylation. Ahmed et al. demonstrated that FOXK1 O-GlcNAcylation promotes recruitment of the deubiquitinase BAP1 to E2F-regulated chromatin and supports transcriptional programs associated with proliferation and oncogenic transformation.81 Importantly, these findings provide direct evidence for a functionally important O-GlcNAc modification of FOXK1 and should not be extrapolated to FOXK2 without additional experimental evidence.

Future studies should determine whether phosphorylation, O-GlcNAcylation, ubiquitination, and other post-translational modifications independently or cooperatively regulate FOXK protein stability, nuclear localization, chromatin occupancy, DNA binding, and recruitment of transcriptional cofactors.

A useful conceptual framework for investigating these relationships is:
Insulin/nutrients → AKT–mTOR/GSK3 signaling → FOXK post-translational modification → subcellular localization → chromatin occupancy → transcriptional output → metabolic phenotype.
Experimental interrogation of each step will be necessary to establish causal relationships and distinguish primary FOXK-dependent events from downstream metabolic consequences.

11.2. Tissue-Specific Functions

FOXK1 and FOXK2 are expressed in multiple tissues, but their biological effects are strongly influenced by cellular context. Experimental studies have demonstrated FOXK-dependent regulation of insulin-responsive metabolism, aerobic glycolysis, mitochondrial function, and proliferation in different cellular systems [4,11,17]. Consequently, FOXK proteins should not be regarded as universal regulators of a single metabolic pathway.

The liver is particularly important because FOXK1 has been directly implicated in insulin-responsive hepatic transcription. Genome-wide analysis demonstrated extensive FOXK1 binding to hepatic promoters and enhancers associated with metabolic and cellular pathways.80 However, insulin signaling is also central to skeletal muscle, adipose tissue, heart, pancreatic islets, and other organs, and it remains uncertain whether the same FOXK regulatory architecture operates quantitatively across these tissues.

Tissue specificity may arise from differences in FOXK1/FOXK2 abundance, chromatin accessibility, transcriptional cofactors, upstream kinase activity, nutrient availability, and post-translational modification. Thus, similar FOXK expression levels do not necessarily imply equivalent transcriptional activity in different tissues.

Recent work in cardiac models further illustrates this context dependence. Cai et al. demonstrated that Foxk1 and Foxk2 promote cardiomyocyte proliferation and cardiac regeneration in experimental models. Both factors directly influenced cell-cycle-associated genes, including Ccnb1 and Cdk1, and promoted HIF1α-associated metabolic reprogramming involving glycolysis and the pentose phosphate pathway.83 These findings demonstrate that FOXK1/FOXK2 can integrate metabolic and cell-cycle regulation outside the liver, although the extent to which this mechanism applies to human cardiac disease remains to be determined.

Similarly, El-Mergawy et al. demonstrated that FOXK2 protein abundance is regulated through FBXO24-dependent ubiquitination in lung epithelial models and that FOXK2 depletion impairs mitochondrial function. FOXK2 was also detected in mitochondrial fractions, raising the possibility of a noncanonical mitochondrial role, however, the molecular targets responsible for this effect remain incompletely characterized [27].

These observations broaden the biological scope of FOXK proteins but should not be interpreted as evidence that identical mechanisms operate in every tissue. Tissue-specific knockout, rescue, and separation-of-function models will therefore be necessary to determine which FOXK functions are conserved and which are organ specific.

Single-cell and spatial transcriptomic approaches could further resolve FOXK-dependent programs among hepatocytes, endothelial cells, fibroblasts, immune cells, and other cellular populations within metabolically active tissues.

An important unresolved issue concerns metabolic disease. In insulin-resistant liver, skeletal muscle, or adipose tissue, FOXK activity may not simply decrease in proportion to impaired insulin signaling. Chronic hyperinsulinemia, altered nutrient flux, inflammation, and changes in AKT, mTOR, and GSK3 signaling may produce tissue-specific FOXK responses.

Longitudinal studies will therefore be required to establish whether altered FOXK activity represents a causal mechanism, an adaptive

response, or a consequence of metabolic dysfunction.

11.3. FOXK1 Versus FOXK2 Specificity

Although FOXK1 and FOXK2 have overlapping biological functions, current evidence does not support considering them completely interchangeable. Both factors can regulate aerobic glycolysis and participate in broader metabolic programs, while experimental depletion of either factor can alter cellular metabolic phenotypes [11,14,].

FOXK1 currently has the strongest direct evidence for participation in hepatic insulin-responsive transcription. The hepatic ChIP-seq study identified extensive FOXK1 occupancy across insulin-responsive regulatory elements and associated FOXK1 with genes involved in metabolism, mitochondrial function, cell-cycle regulation, and other biological processes [9]. FOXK1 is also an established downstream effector of mTORC1-dependent metabolic regulation. He et al. demonstrated that suppression of mTORC1 increases GSK3-dependent FOXK1 phosphorylation, promotes 14-3-3 binding and nuclear exclusion, and reduces FOXK1-dependent metabolic transcription [17].

FOXK2, in contrast, has emerging evidence for specialized functions involving mitochondrial regulation, protein turnover, cellular stress, and context-dependent metabolic remodeling. El-Mergawy et al. showed that FOXK2 is targeted for ubiquitin-mediated degradation by the SCF-E3 ligase component FBXO24 and that experimental FOXK2 depletion impairs mitochondrial respiratory function in lung epithelial models [27].

The distinction between FOXK1 and FOXK2 is further illustrated by nutrient-sensitive O-GlcNAcylation. Ahmed et al. demonstrated that functionally important O-GlcNAcylation of FOXK1 promotes BAP1 recruitment to E2F-regulated chromatin and supports proliferative and oncogenic transcription [14]. This study provides direct evidence for FOXK1-specific nutrient-sensitive regulation but does not establish an equivalent mechanism for FOXK2.

Future investigations should compare FOXK1 and FOXK2 in matched cellular and physiological systems rather than inferring specificity from studies conducted in unrelated tissues. Parallel knockout, rescue, domain-swap, and separation-of-function experiments could determine whether functional differences arise from DNA occupancy, protein stability, post-translational modification, subcellular localization, or recruitment of distinct cofactors.

Integrated analyses of chromatin occupancy, protein–protein interactions, phosphorylation, O-GlcNAcylation, ubiquitination, transcriptional output, and metabolic flux would provide a more rigorous framework for determining which FOXK1/FOXK2 functions are redundant and which are factor specific.

Overall, current evidence supports viewing FOXK1 and FOXK2 as a partially redundant but functionally specialized transcription-factor pair, rather than as identical metabolic regulators.

11.4. Human Genetic and Clinical Evidence

A major limitation of the FOXK literature is the relatively limited amount of direct human genetic and clinical evidence compared with the extensive mechanistic evidence generated in experimental models. Current evidence includes cultured cells, hepatocyte systems, animal models, and selected human cellular models, but these studies do not establish that FOXK dysregulation is a major determinant of human metabolic disease [4,9,11,32].

Human genetic studies could provide an independent assessment of the physiological importance of FOXK1 and FOXK2. Genome-wide association studies, exome sequencing, whole-genome sequencing, and population-based analyses should be examined for variants in FOXK1, FOXK2, and their regulatory regions associated with insulin sensitivity, type 2 diabetes, obesity, dyslipidemia, hepatic steatosis, or related metabolic phenotypes. However, the absence of strong common-variant associations would not exclude biological relevance. Transcription factors may influence disease through tissue-specific regulation, rare variants, epigenetic mechanisms, altered protein stability, or post-translational modifications rather than through common coding variants.

Functional genomic analyses should therefore be integrated with detailed clinical phenotyping. FOXK1/FOXK2 expression, protein abundance, subcellular localization, post-translational modification, chromatin occupancy, and downstream transcriptional signatures could be assessed in human liver, skeletal muscle, adipose tissue, and tumor specimens.

Cancer represents another important area for investigation. FOXK1 has been linked to proliferative transcriptional programs, including E2F-associated regulation, whereas FOXK2 has demonstrated context-dependent effects on mitochondrial function and cellular metabolism [14,27]. In addition, experimental evidence in cervical cancer indicates that FOXK2 can promote fatty-acid oxidation and tumor progression through an mTOR/DRP1-associated signaling axis.85 These findings provide mechanistic hypotheses but should not yet be interpreted as evidence of a universal FOXK2 mechanism across human cancers.

Importantly, FOXK expression alone may be insufficient as a biomarker of pathway dependence. Future biomarker development should consider composite measures incorporating protein abundance, subcellular localization, post-translational modification, FOXK target-gene expression, chromatin occupancy, and metabolic phenotype.

Longitudinal clinical studies will also be valuable. Sampling before and after weight-loss interventions, insulin-sensitizing therapies, mTOR-directed treatment, or anticancer therapy could determine whether FOXK activity changes dynamically in response to metabolic or pharmacological interventions.

At present, FOXK1/FOXK2 should therefore be considered experimentally supported biological regulators with emerging

clinical relevance, rather than established clinical biomarkers or validated therapeutic targets.

11.5. Integration of Transcriptomics, ChIP-seq, Proteomics, and Metabolomics

The complexity of FOXK regulation cannot be adequately resolved using a single experimental platform. FOXK activity is influenced by signaling-dependent phosphorylation, protein stability, subcellular localization, chromatin occupancy, transcriptional cofactors, and metabolic feedback [11,14,17,27].

Transcriptomic analyses can identify genes whose expression changes following FOXK perturbation but cannot reliably distinguish direct from indirect targets. ChIP-seq, CUT&RUN, and CUT&Tag can identify FOXK-associated genomic regions, whereas ATAC-seq can determine whether these sites occur within accessible chromatin. Integration of these datasets with nascent RNA sequencing would provide stronger evidence for identifying transcriptionally functional FOXK targets.

Proteomic and phosphoproteomic approaches are particularly important because FOXK activity is regulated by signaling-dependent phosphorylation [4,17]. Future studies should determine how insulin, IGF-1, nutrient deprivation, mTOR inhibition, and GSK3 modulation alter FOXK phosphorylation and protein interactions in a tissue-specific manner.

O-GlcNAcylation represents an additional nutrient-sensitive regulatory layer. The demonstration that FOXK1 O-GlcNAcylation promotes BAP1 recruitment and E2F-associated transcription provides a potential molecular connection between nutrient flux and chromatin regulation [14]. Comparative phosphoproteomic and O-GlcNAc proteomic studies could determine whether these modifications cooperate, compete, or act sequentially.

Metabolomics and metabolic-flux analysis are equally important. Changes in metabolic gene expression do not necessarily correspond to equivalent changes in pathway activity. Stable-isotope tracing using substrates such as ^13C-glucose and ^13C-palmitate could determine how FOXK perturbation alters glycolytic flux, lactate production, pyruvate oxidation, tricarboxylic-acid-cycle activity, fatty-acid oxidation, and anabolic biosynthesis.

Mitochondrial phenotyping should include extracellular-flux measurements, substrate-specific respiration, ATP production, mitochondrial membrane potential, reactive oxygen species, mitochondrial morphology, and respiratory-chain function. Such measurements would help distinguish transcriptional changes from genuine alterations in mitochondrial bioenergetics.

A comprehensive experimental framework could therefore be represented as:
FOXK1/FOXK2 genetic perturbation
↓.
RNA-seq + nascent RNA-seq
↓
ChIP-seq/CUT&RUN/CUT&Tag + ATAC-seq
↓
Proteomics + phosphoproteomics + O-GlcNAc proteomics
↓
Metabolomics + stable-isotope tracing
↓
Mitochondrial respirometry + imaging
↓
In vivo physiological phenotyping
↓
Validation in human tissues and disease cohorts

This integrated strategy would provide a systems-level understanding of how insulin and nutrient signals are translated into FOXK-dependent transcriptional and metabolic states.
Publicly available chromatin and transcriptomic datasets also provide opportunities for independent validation and cross-study integration. The hepatic FOXK1 dataset generated by Allu et al. can be integrated with independent insulin-response, FOXO1, and metabolic-disease datasets to identify conserved regulatory programs.80 Similarly, FOXK1/FOXK2 chromatin and transcriptomic datasets from other tissues can be compared with cancer and metabolic-disease cohorts to distinguish conserved pathways from tissue-specific signatures.

Ultimately, the field should progress from descriptive associations toward causal regulatory-network reconstruction. The key objective is not merely to determine where FOXK1 or FOXK2 binds, but to establish how insulin- and nutrient-dependent signaling modifies FOXK activity, how these changes affect chromatin occupancy and transcription, and how transcriptional changes translate into metabolic flux, mitochondrial function, cellular behavior, and tissue physiology.

12. Conclusion

FOXK1 and FOXK2 have emerged as important nutrient-responsive transcription factors that expand the conventional view of insulin-regulated gene expression beyond the classical FOXO-centered framework. Experimental studies demonstrate that insulin promotes redistribution of FOXK1 and FOXK2 toward the nucleus, whereas GSK3-dependent phosphorylation contributes to their cytoplasmic retention under basal conditions. This behavior contrasts with FOXO1, whose phosphorylation by AKT promotes nuclear exclusion after insulin stimulation [1]. These reciprocal responses suggest that insulin can simultaneously suppress fasting-associated transcriptional programs mediated by FOXO1 while activating distinct FOXK-dependent transcriptional programs involved in nutrient utilization and cellular adaptation.

The available evidence indicates that FOXK1 and FOXK2 function as transcriptional integrators rather than as regulators of a single metabolic pathway. Both factors can promote aerobic glycolysis by increasing the expression of glycolytic enzymes, including hexokinase 2, phosphofructokinase, pyruvate kinase, and lactate dehydrogenase, while also increasing PDK1/PDK4 activity and thereby limiting pyruvate oxidation through inhibition of the

pyruvate dehydrogenase complex.[2] At the same time, FOXK1/FOXK2 perturbation alters lipid metabolism, mitochondrial respiration, and cellular energy metabolism [1]. These apparently divergent effects are more appropriately interpreted as evidence of metabolic flexibility: FOXK proteins can influence the relative utilization of glucose and lipid substrates according to cellular and environmental requirements rather than functioning exclusively as either glycolytic or oxidative regulators.

FOXK1 appears particularly important for hepatic insulin-responsive transcription. Genome-wide ChIP-seq analysis demonstrated that FOXK1 occupies more than 4,000 proximal promoters and enhancers in hepatic chromatin and that insulin increases FOXK1 occupancy at approximately 75% of these sites. These regions include genes involved in glucose and mitochondrial metabolism, cell-cycle regulation, steroid biosynthesis, autophagy, and cellular senescence. FOXK1 binding also overlaps with selected FOXO1- and insulin-receptor-associated transcriptional programs, supporting a role for FOXK1 as a component of the hepatic insulin-responsive gene-regulatory network [3]. Independent work using promoter-based approaches similarly identified FOXK1 as an insulin-responsive transcription factor contributing to regulation of hepatocyte glucose production [4].

The relationship between FOXK proteins and mTOR signaling further illustrates their role as nutrient-responsive transcriptional regulators. mTORC1 suppresses GSK3-dependent phosphorylation of FOXK1, thereby limiting 14-3-3 binding and nuclear exclusion and promoting FOXK1-dependent metabolic transcription. FOXK1 can consequently contribute to mTORC1-associated metabolic reprogramming through regulation of HIF1α and glycolytic and anabolic pathways [5]. This mechanism provides an important molecular connection between nutrient sensing, kinase signaling, transcription-factor localization, and metabolic adaptation.

Additional evidence demonstrates that FOXK-dependent transcription is influenced by nutrient-sensitive post-translational regulation. O-GlcNAcylation of FOXK1 promotes recruitment of the deubiquitinase BAP1 to E2F-regulated chromatin and enhances proliferative transcriptional programs, linking nutrient-sensitive protein modification to transcriptional control and oncogenic phenotypes [6]. Importantly, this mechanism is currently demonstrated specifically for FOXK1 and should not be extrapolated to FOXK2 without direct experimental evidence.

FOXK1 and FOXK2 should therefore not be considered functionally identical. Although they share substantial sequence similarity and overlapping roles in metabolic regulation, differences in protein stability, post-translational modification, transcriptional cofactors, genomic occupancy, and tissue context may generate distinct biological effects [1,2]. FOXK1 currently has stronger evidence for direct hepatic insulin-responsive transcription, whereas FOXK2 has emerging evidence for specialized roles in mitochondrial regulation and cellular stress responses. In lung epithelial models, FOXK2 is subject to ubiquitin-dependent degradation mediated by the SCF-E3 ligase component FBXO24, and FOXK2 depletion impairs mitochondrial function, FOXK2 has also been detected in mitochondrial fractions [7]. These findings support a potential role for FOXK2 in mitochondrial regulation but should not be generalized to all tissues without additional evidence.

Recent studies further demonstrate that both FOXK1 and FOXK2 can regulate cell proliferation in a tissue-specific manner. In experimental cardiac models, FOXK1 and FOXK2 promote cardiomyocyte proliferation by activating cell-cycle regulators including CCNB1 and CDK1 and by promoting HIF1α-associated metabolic reprogramming [8]. These findings reinforce the concept that FOXK proteins can couple metabolic state with proliferative capacity. However, the cardiac findings primarily derive from experimental models and should not be interpreted as evidence of an equivalent mechanism in human cardiac disease.

The translational implications of FOXK biology are promising but remain preliminary. FOXK1/FOXK2 are attractive candidate regulatory nodes because they intersect with pathways controlling insulin signaling, glycolysis, lipid metabolism, mitochondrial function, proliferation, and cellular adaptation. However, their physiological functions in normal tissues create substantial challenges for nonspecific systemic inhibition. Moreover, FOXK1 and FOXK2 may exert different effects depending on tissue and disease context. Therefore, FOXK proteins should currently be considered **candidate molecular regulators and potential biomarkers of metabolic dependency**, rather than clinically validated therapeutic targets.

Disease-specific metabolic dependencies may nevertheless provide opportunities for therapeutic intervention. For example, FOXK2 has been implicated in fatty-acid metabolic remodeling and cervical cancer progression through an mTOR/DRP1-associated mechanism involving CPT1A and fatty-acid oxidation [9]. Such findings provide proof-of-concept that FOXK-dependent metabolic pathways may become therapeutically relevant in selected tumor contexts. However, these observations remain preclinical and should not be interpreted as evidence that direct FOXK2 inhibition is currently clinically feasible.

Several priorities should guide future investigation. First, direct FOXK1/FOXK2 transcriptional targets must be distinguished from secondary gene-expression changes using integrated chromatin-occupancy and nascent-transcription approaches. Second, tissue-specific and disease-specific FOXK functions should be defined using conditional genetic models and human tissues. Third, FOXK1 and FOXK2 specificity should be investigated through matched knockout, rescue, and biochemical experiments. Fourth, human genetic and clinical studies are needed to determine whether FOXK activity contributes meaningfully to metabolic disease or predicts therapeutic response. Finally, integration of transcriptomics, chromatin profiling, proteomics, phosphoproteomics, O-GlcNAc proteomics, metabolomics, and metabolic-flux analysis will be necessary to reconstruct causal FOXK regulatory networks.

Taken together, the current evidence supports a model in which insulin and nutrient availability regulate FOXK-dependent transcription through interconnected signaling and post-translational mechanisms:

The reciprocal regulation of FOXK and FOXO1 therefore provides a broader conceptual framework for understanding how insulin converts an extracellular hormonal signal into coordinated transcriptional responses. FOXK1 and FOXK2 are best viewed as **context-dependent transcriptional integrators that help coordinate glucose utilization, lipid metabolism, mitochondrial function, and cellular proliferation according to nutrient and signaling conditions**. Defining the precise physiological and pathological boundaries of these functions will be essential before FOXK-directed strategies can progress from mechanistic research toward clinical translation and also in figure 3 shown in diagrammatic way [33].

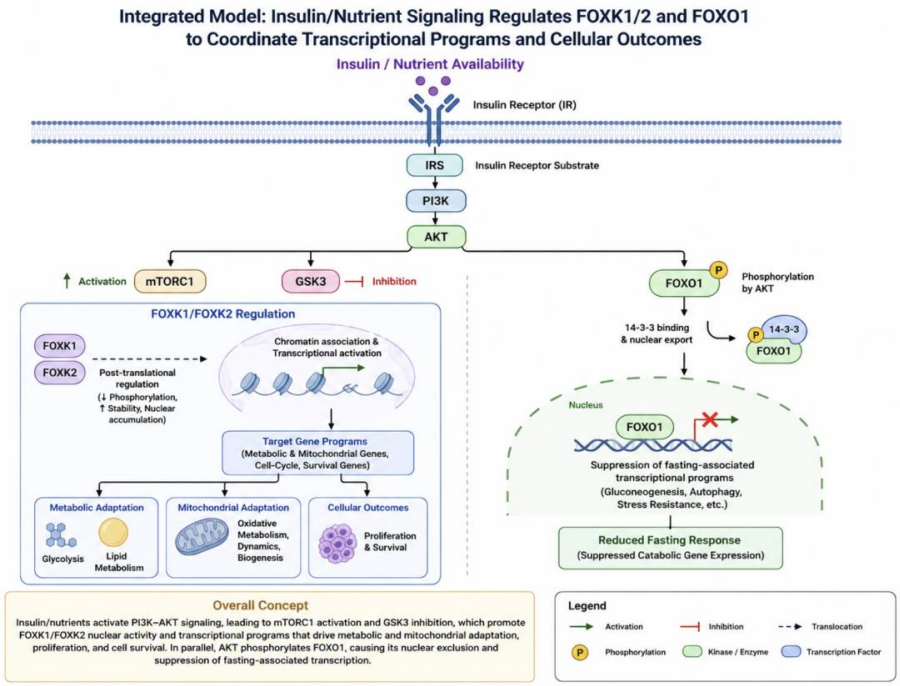

Figure 3: Integrated Model: Insulin/Nutrient Signalling Regulates FOXO1 to Coordinate Transcriptional Programs and Cellular Outcomes

References

1. Petersen, M. C., & Shulman, G. I. (2018). Mechanisms of insulin action and insulin resistance. *Physiological reviews, 98*(4), 2133-2223.
2. Taniguchi, C. M., Emanuelli, B., & Kahn, C. R. (2006). Critical nodes in signalling pathways: insights into insulin action. *Nature reviews Molecular cell biology, 7*(2), 85-96.
3. Haeusler, R. A., Kaestner, K. H., & Accili, D. (2010). FoxOs function synergistically to promote glucose production. *Journal of Biological Chemistry, 285*(46), 35245-35248.
4. Sakaguchi, M., Cai, W., Wang, C. H., Cederquist, C. T., Damasio, M., Homan, E. P., ... & Kahn, C. R. (2019). FoxK1 and FoxK2 in insulin regulation of cellular and mitochondrial metabolism. *Nature Communications, 10*(1), 1582.
5. Samuel, V. T., & Shulman, G. I. (2016). The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. *The Journal of clinical investigation, 126*(1), 12-22.
6. Hatting, M., Tavares, C. D., Sharabi, K., Rines, A. K., & Puigserver, P. (2018). Insulin regulation of gluconeogenesis. *Annals of the New York Academy of Sciences, 1411*(1), 21-35.
7. Katoh, M., & Katoh, M. (2004). Human FOX gene family. *International journal of oncology, 25*(5), 1495-1500.
8. Okino, Y., Machida, Y., Frankland-Searby, S., & Machida, Y. J. (2015). BRCA1-associated protein 1 (BAP1) deubiquitinase antagonizes the ubiquitin-mediated activation of FoxK2 target genes. *Journal of Biological Chemistry, 290*(3), 1580-1591.
9. Allu, P. K., Cardamone, M. D., Gomes, A. S., Dall'agnese, A., Cederquist, C., Pan, H., ... & Kahn, C. R. (2023). FoxK1 associated gene regulatory network in hepatic insulin action and its relationship to FoxO1 and insulin receptor mediated transcriptional regulation. *Molecular Metabolism, 78*, 101825.
10. Wang, L., Liu, Q., Kitamoto, T., Hou, J., Qin, J., & Accili, D. (2019). Identification of insulin-responsive transcription factors that regulate glucose production by hepatocytes. *Diabetes, 68*(6), 1156-1167.
11. Sukonina, V., Ma, H., Zhang, W., Bartesaghi, S., Subhash, S., Heglind, M., ... & Enerbäck, S. (2019). FOXK1 and FOXK2 regulate aerobic glycolysis. *Nature, 566*(7743), 279-283.
12. UniProt Consortium. FOXK1: Forkhead box protein K1

[Homo sapiens]. UniProtKB P85037. Available from: UniProt FOXK1.
13. Myatt, S. S., & Lam, E. W. F. (2007). The emerging roles of forkhead box (Fox) proteins in cancer. *Nature Reviews Cancer, 7*(11), 847-859.
14. Ahmed, O., Masclef, L., Iannantuono, N., Gagnon, J., Gushul-Leclaire, M., Boulay, K., ... & Affar, E. B. (2025). O-GlcNAcylation of FOXK1 co-opts BAP1 to orchestrate the E2F pathway and promotes oncogenesis. *Nature Communications, 16*(1), 5959.
15. Shi, X., & Garry, D. J. (2012). Sin3 interacts with Foxk1 and regulates myogenic progenitors. *Molecular and cellular biochemistry, 366*(1), 251-258.
16. Shi, X., Seldin, D. C., & Garry, D. J. (2012). Foxk1 recruits the Sds3 complex and represses gene expression in myogenic progenitors. *Biochemical Journal, 446*(3), 349-357.
17. He, L., Gomes, A. P., Wang, X., Yoon, S. O., Lee, G., Nagiec, M. J., ... & Blenis, J. (2018). mTORC1 promotes metabolic reprogramming by the suppression of GSK3-dependent Foxk1 phosphorylation. *Molecular cell, 70*(5), 949-960.
18. Saltiel, A. R. (2021). Insulin signaling in health and disease. *The Journal of clinical investigation, 131*(1).
19. Boucher, J., Kleinridders, A., & Kahn, C. R. (2014). Insulin receptor signaling in normal and insulin-resistant states. *Cold Spring Harbor perspectives in biology, 6*(1), a009191.
20. Yu, M., Yu, H., Mu, N., Wang, Y., Ma, H., & Yu, L. (2022). The function of FoxK transcription factors in diseases. *Frontiers in Physiology, 13*, 928625.
21. Okino, Y., Machida, Y., Frankland-Searby, S., & Machida, Y. J. (2015). BRCA1-associated protein 1 (BAP1) deubiquitinase antagonizes the ubiquitin-mediated activation of FoxK2 target genes. *Journal of Biological Chemistry, 290*(3), 1580-1591.
22. Li, L., Gong, M., Zhao, Y., Zhao, X., & Li, Q. (2017). FOXK1 facilitates cell proliferation through regulating the expression of p21, and promotes metastasis in ovarian cancer. *Oncotarget, 8*(41), 70441.
23. Wang, P., Zhai, G., & Bai, Y. (2018). Values of miR-34a and miR-218 expression in the diagnosis of cervical cancer and the prediction of prognosis. *Oncology Letters, 15*(3), 3580-3585.
24. van der Heide, L. P., Wijchers, P. J., von Oerthel, L., Burbach, J. P. H., Hoekman, M. F., & Smidt, M. P. (2015). FoxK2 is required for cellular proliferation and survival. *Journal of cellular physiology, 230*(5), 1013-1023.
25. Sakaguchi, M., Cai, W., Wang, C. H., Cederquist, C. T., Damasio, M., Homan, E. P., ... & Kahn, C. R. (2019). FoxK1 and FoxK2 in insulin regulation of cellular and mitochondrial metabolism. *Nature Communications, 10*(1), 1582.
26. Liao, D., Zeng, S., Li, C., Yao, Y., Guo, M., Cui, Y., & Huang, H. (2025). FOXK2 regulates fatty acid metabolism and promotes cervical cancer progression by activating the mTOR/DRP1 signaling axis. *Frontiers in cell and developmental biology, 13*, 1615454.
27. El-Mergawy, R., Chafin, L., Ovando-Ricardez, J. A., Rosas, L., Tsai, M., Rojas, M., ... & Mallampalli, R. K. (2024). FOXK2 targeting by the SCF-E3 ligase subunit FBXO24 for ubiquitin mediated degradation modulates mitochondrial respiration. *Journal of Biological Chemistry, 300*(6).
28. He, L., Gomes, A. P., Wang, X., Yoon, S. O., Lee, G., Nagiec, M. J., ... & Blenis, J. (2018). mTORC1 promotes metabolic reprogramming by the suppression of GSK3-dependent Foxk1 phosphorylation. *Molecular cell, 70*(5), 949-960.
29. Xing, X., Que, X., Zheng, S., Wang, S., Song, Q., Yao, Y., & Zhang, P. (2024). Emerging roles of FOXK2 in cancers and metabolic disorders. *Frontiers in oncology, 14*, 1376496.
30. Fujinuma, S., Nakatsumi, H., Shimizu, H., Sugiyama, S., Harada, A., Goya, T., ... & Nakayama, K. I. (2023). FOXK1 promotes nonalcoholic fatty liver disease by mediating mTORC1-dependent inhibition of hepatic fatty acid oxidation. *Cell reports, 42*(5).
31. Bowman, C. J., Ayer, D. E., & Dynlacht, B. D. (2014). Foxk proteins repress the initiation of starvation-induced atrophy and autophagy programs. *Nature cell biology, 16*(12), 1202-1214.
32. Cai, D., Liu, C., Li, H., Wang, C., Bai, L., Feng, J., ... & Nie, Y. (2025). Foxk1 and Foxk2 promote cardiomyocyte proliferation and heart regeneration. *Nature Communications, 16*(1), 2877.
33. Yu, M., Yu, H., Mu, N., Wang, Y., Ma, H., & Yu, L. (2022). The function of FoxK transcription factors in diseases. *Frontiers in Physiology, 13*, 928625.