

Minireview

The FoxO transcription factors and metabolic regulation

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Abstract Forkhead transcription factors FoxOs are conserved beyond species and regulated by insulin signaling pathway. FoxOs have diverse functions on differentiation, proliferation and cell survival. In calorie restriction (CR) or starvation, FoxOs are in nucleus, active transcriptionally, and increase hepatic glucose production, decrease insulin secretion, increase food intake and cause degradation of skeletal muscle for supplying substrates for glucose production. However, even in insulin resistance due to excessive calorie intake, FoxOs are active and causes type 2 diabetes and hyperlipidemia. The understanding of molecular mechanism how FoxOs affect glucose or lipid metabolism will shed light on the novel therapy of type 2 diabetes and the metabolic syndrome.

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1. Introduction

In nematode *Caenorhabditis elegans* (*C. elegans*), in response to increased levels of a secreted pheromone as well as food deprivation during early larval stage (L1), worms can enter an alternative developmental mode and form dauer larvae [1,2]. DAF-2 (a homolog of the mammalian insulin receptor) pathway regulates a state of dauer. Analyses of several mutant *C. elegans* identified several genes, which includes *age-1* (a homolog of the catalytic subunit of mammalian phosphatidylinositol 3-OH kinase) and *akt*. Loss-of-function mutants of these genes showed extended lifespan and/or constitutive dauer formation [3]. *Daf-16* was initially isolated as a gene that caused a dauer defective phenotype when mutated and found that it encodes a forkhead/HNF3-related transcription factor [3,4]. The closest human orthologues are *FKHR* (*FOXO1*), *FKHR-L1* (*FOXO3A*), and *AFX* (*FOXO4*) [5]. It was speculated that they might act downstream of insulin signaling and be deregulated in diabetes.

Initially, a subset of *FOXO* genes has been identified with disorders like rhabdomyosarcomas and tumorigenesis. A form of rhabdomyosarcoma is caused by a translocation between

chromosome 2 or chromosome 1 and chromosome 13 [t(1,13) or t(2,13)], which leads to fusion of the *PAX7* or *PAX3* gene with *FOXO1*. *FOXO3A* and *FOXO4* are fusion partners of MLL in acute myeloid leukemias (AMLs) associated with t(6;11)(q21;q23) or t(X;11)(q13;q23), respectively [6]. The winged helix/forkhead class of transcription factors is characterized by a 100-amino acid, monomeric DNA-binding domain. The DNA-binding domain folds into a variant of the helix–turn–helix motif and is made up of three α helices and two characteristic large loops, or wings. Therefore, the DNA-binding motif has been named the winged helix DNA-binding domain [7]. Until the present time, over 100 members of this gene family have been identified. In 2000, in order to standardize the nomenclature for these proteins, Fox (Forkhead box) was adopted as the unified symbol for all chordate winged helix/forkhead transcription factors [8]. Since 1999, many FoxO-target genes and -functions were identified. These studies have been reviewed extensively elsewhere [5,6].

In the present review, we will summarize metabolic effects of FoxOs in insulin responsive tissues and discuss how FoxOs regulate glucose and lipid metabolism.

2. Regulation of FoxOs' activity

2.1. Regulation of phosphorylation and subcellular localization of FoxOs by PI-3kinase-dependent way

After insulin, IGF-1 or other growth factors bind to their tyrosine kinase receptors and activate phosphoinositide kinase (PI3K), several serine/threonine kinases, which include the Akt family protein kinases and the related serum and glucocorticoid inducible kinase (SGK) are activated [9]. FoxOs have three consensus phosphorylation sites (RXXRXXS/T) of Akt (threonine 24, serine 253 and serine 316 in murine FoxO1) although DAF-16 in *C. elegans* has four consensus Akt phosphorylation sites [10–15]. Serine 253 acts as a gatekeeper site for phosphorylation of FoxO1 and phosphorylation of Serine 253 is needed for subsequent phosphorylation of Threonine 24 and Serine 316 [12]. Not only Akt phosphorylates these sites but also, in some cell lines, another kinase, SGK, phosphorylates FoxOs. Each kinase has preferential sites for phosphorylation of FoxOs. Akt phosphorylates Serine 253 and SGK phosphorylates Serine 316 preferentially. Threonine 24 is phosphorylated by both kinases [10] (Fig. 1).

Phosphorylation of three Akt/SGK phosphorylation sites causes changes of subcellular localization of FoxOs [10,15]. In inactivated state of Akt/SGK, FoxOs are in nucleus and

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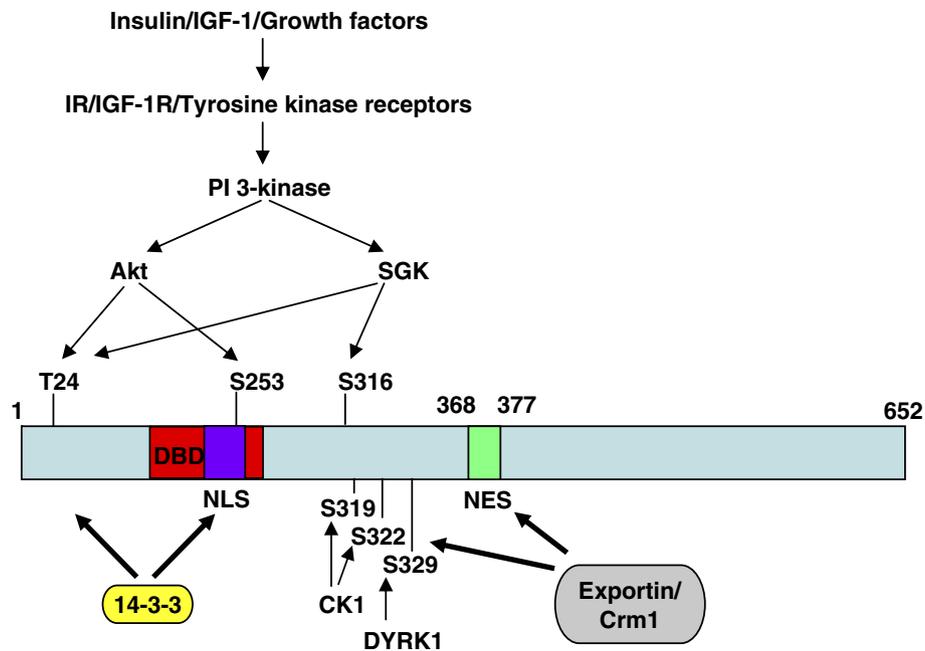


Fig. 1. Phosphorylation and regulation of nuclear export of FoxO1. Insulin or growth factor binds to IR or other tyrosine kinase receptor and leads to activation of PI3K pathway. Akt/SGK phosphorylates Threonine 24, Serine 253, and Serine 316 of FoxO1. The chaperone protein, 14-3-3, binds to phosphorylated Threonine 24 and Serine 253 and promotes the nuclear export of FoxO1. The 14-3-3 binding to NLS also prevents nuclear import of FoxO1 and these events leads to exposure of NES and interaction with Exportin/Crm1. Phosphorylation of Serine 319 and 322 by CK1 accelerates FoxO1 relocalization by interaction with Exportin/Crm1.

believed to activate or inhibit gene expression of their target genes. After stimulation with several growth factors, Akt/SGK is activated and phosphorylates FoxOs and causes the export of FoxOs to the cytoplasm. Several mechanisms about shuttling of FoxOs between nucleus and cytoplasm have been described. FoxOs have a nuclear export sequence (NES) in the conserved C-terminal region [15]. Furthermore, a chaperone molecules, 14-3-3 protein, binds to phosphorylated FoxOs [10,16]. 14-3-3 binding may decrease the ability of FoxOs to bind DNA, releasing FoxOs from DNA [17]. 14-3-3 binding to FoxOs may promote the nuclear export of FoxOs by inducing a conformational change in FoxO molecules that would expose the NES and allow interaction with Exportin/Crm1 [16]. 14-3-3 binding to FoxOs may also prevent the nuclear import of FoxOs by masking FoxO nuclear localization signal (NLS) [18,19] (Fig. 1).

FoxO6, which is another member of FoxOs, has been identified recently. FoxO6 has only two Akt/SGK phosphorylation sites (Threonine 26 and Serine 184 in mouse FoxO6) and stays in nucleus mostly. However, phosphorylation of these two sites decreases transcriptional activity of FoxO6 [20].

However, only nuclear localization may not give FoxOs full transcriptional activity because studies in *C. elegans* demonstrated that the DAF-2 pathway still inhibited dauer formation and lifespan extension even when the consensus Akt sites on DAF-16a are not phosphorylated [21]. Further analyses of regulation of FoxOs' activities might be needed.

2.2. Regulation of FoxOs by other signaling pathways

It has been reported that other kinases phosphorylate several different sites in response to insulin and growth factors. Casein kinase 1 (CK1) phosphorylates Serine 319 (in mouse FoxO1) and Serine 322 in FoxO1 [22] and the dual tyrosine

phosphorylated regulated kinase 1 (DYRK1) phosphorylates Serine 329 [23]. Phosphorylation of Serine 319 and 322 may also contribute to acceleration of FoxO relocalization to cytoplasm by increasing the interaction between FoxOs and the export machinery (Ran and Exportin/Crm1) [22]. Phosphorylation of Serine 329 by DYRK1 also accelerates nuclear export and inhibition of FoxOs' transcriptional activity [23].

The c-Jun N-terminal kinase (JNK) positively regulates FoxOs activity in *C. elegans* and *Drosophila*. Activation of JNK pathway extends life span of both species [24,25]. In *C. elegans*, JNK-1 phosphorylates DAF-16. The phosphorylation was observed only with the N-terminal (amino acids 83–307) fragment of DAF-16. Furthermore, it has been demonstrated that JNK-1 directly interacts with, phosphorylates, enhances nuclear localization of DAF-16, and induces its target gene expression [24]. Indeed, low levels of oxidative stress generated by treatment with H₂O₂ activates the small GTPase Ral and induce a JNK-dependent phosphorylation of Threonine 447 and Threonine 451, nuclear translocation, and transcriptional activation of FOXO4 [26].

One of null-mutations in *C. elegans*, *bar-1*, which is homolog of the β -catenin in mammalian, was found as defective mutant of starvation-induced dauer development [27]. β -catenin is a multifunctional protein that mediates Wnt signaling by binding to members of the T-cell factor (TCF) family of transcription factors [28,29]. BAR-1 may increase dauer formation and life span by increasing DAF-16 activity. The physical interaction between FoxOs and β -catenin is conserved. β -catenin enhances FoxOs' transcriptional activity. Insulin stimulation causes nuclear export of FoxOs and inhibits them. At that time, β -catenin induces cell proliferation through activation of TCFs. In contrast, oxidative stress induces nuclear localization of FoxOs and β -catenin activates FoxOs' transcriptional

activity and causes cell cycle arrest [30]. The previous findings that both Wnt signaling pathway and FoxO1 have been shown to inhibit adipocyte differentiation suggest a speculative hypothesis that these two signaling pathway may converge on promoter regions of their target genes during adipocyte differentiation [31–33].

2.3. Regulation of FoxOs by CBP/p300-mediated acetylation and Sirt1-mediated deacetylation

Calorie restriction (CR) was first described as a reduction in food intake in laboratory rodents of between 20% and 40% of ad libitum levels that would extend their life span by up to 50% [35]. The effects of CR are beginning with an acute phase upon imposition of the diet followed by an adaptive period of several weeks to reach a stable, altered physiological state. A lower body temperature, lower blood glucose and insulin levels, and reduced body fat and weight characterize this altered state [34]. CR regulates Sirt1 expression level and activity. Sirt1 is one of the seven mammalian Sir2 ortholog, which is a nicotinamide adenine dinucleotide (NAD)-dependent histone deacetylase [35,36]. Mutant strains of *C. elegans* with extra copies of Sir2.1 have increased longevity and Daf16 is epistatic to Sir2.1 gain-of-function [37]. Sirt1 has several roles in mammalian physiology. In white adipose tissue, Sirt1 was shown to inhibit adipogenesis in precursor cells and reduce fat storage in differentiated cells through inhibition of Ppar γ by Sirt1 docking with its negative cofactors NcoR and SMRT [38]. In pancreatic β -cell, Sirt1 is a positive regulator of insulin secretion. Lowering Sirt1 in the insulinoma cells activated transcription of the *uncoupling protein 2 gene (Ucp-2)*, which encodes a mitochondrial membrane protein that might uncouple ATP synthesis from respiration. Sirt1 suppresses *Ucp-2* expression in pancreatic β -cells and increases the efficiency of ATP synthesis in response to glucose and finally positively regulates insulin secretion [39,40]. Sirt1 was also demonstrated to protect β -cells against oxidative stress in a mechanism proposed to involve deacetylation of FoxO1 [41]. Furthermore, Sirt1 is also involved in hepatic gluconeogenesis. Sirt1 binds to and deacetylates Ppar γ coactivator Pgc-1 α and FoxO1 and activates them, resulting in an increase in glucose production [42,43].

FoxOs bind to the transcriptional coactivator CBP (CREB-binding protein) and to p300, thus providing a connection between these transcriptional regulators and the basal transcriptional machinery [44–46]. CBP, p300, and PCAF directly acetylate FoxOs at several conserved lysine residues [47,47, 48]. It has been reported that the acetylation of FoxOs by CBP suppresses FoxOs' transcriptional activity [49] and inhibits FoxO's binding to DNA [50]. Although the effects of deacetylation of FoxOs by Sirt1 vary depending on FoxO-target genes, Sirt1 has been reported to enhance FoxO's transcriptional activity on their target genes [47,49,46]. As a whole, Sirt1 showed that it deacetylates FoxOs and increases the stress resistance of cells by inhibiting apoptosis and increasing stress resistance [5]. Other than Lysine residues in FoxOs can regulate these events. FoxOs have the conserved LXXLL motif in their C-terminal region. The LXXLL motif of FoxO1 appears to be important for its binding to Sirt1. Although it has not been elucidated completely how the LXXLL motif of FoxO1 mediates the binding to Sirt, the disruption of the LXXLL motif of FoxO1 inhibits transcriptional activity of FoxO1 and enhance acetylation of FoxO1 in liver [51].

2.4. Ubiquitination and degradation of FoxOs

Another regulation of FoxOs' activity is the ubiquitination-mediated degradation of FoxOs in the 26S proteasome. Matsuzaki et al. has reported that insulin decreased FoxO1 protein levels due to ubiquitination and degradation in the 26S proteasome in the PI3-kinase-dependent manner in HepG2 cells [52]. Furthermore, Aoki et al. demonstrated that FoxO1 expression levels were decreased in chicken embryo fibroblast (CEF) transformed by PI3-kinase and Akt and suggested that phosphorylation-mediated degradation of FoxO1 may play a role in oncogenic transformation by PI3-kinase and Akt [53].

Many tumor suppressors can be degraded by the ubiquitin pathway in human cancer. P27^{KIP1}, p130 and p57^{KIP2} have been shown to be targeted by the F-box motif in Skp2, an oncogenic subunit of the Skp1/Cul1/F-box protein ubiquitin complex, for degradation [54–57]. Skp2 interacts with, ubiquitinates, and promotes the degradation of FOXO1 with Akt-specific phosphorylation of Serine 256 [58]. Therefore, Skp2 suppresses tumor-suppressor effects of FoxO1. Furthermore, Skp2 is also involved in regulation of glucose metabolism, which includes the regulation of number of pancreatic β -cell, insulin secretion and adipocyte proliferation during the development of obesity [59]. Therefore, ubiquitination and degradation of FoxO1 by Skp2 may contribute to the onset of insulin resistance and type 2 diabetes.

3. Knockout mice of FoxOs

In order to elucidate physiological roles of each FoxO in vivo, we have to see phenotypes of each FoxO-knockout mouse (Table 1). However, we have to mind whether each FoxO has the same physiological roles or not, whether each FoxO can compensate another FoxO or not.

3.1. FoxO1 knockout mice

FoxO1-knockout embryos died on embryonic day 10.5 as a consequence of incomplete vascular development. At E9.5, *FoxO1*^{-/-} yolk sacs lacked well-developed blood vessels. The primary defect in *FoxO1*^{-/-} embryos and yolk sacs appears to be a disruption of normal vascular formation. Indeed,

Table 1
Phenotypes of FoxOs knockout mice

Knockout mice	Phenotypes	References
<i>FoxO1</i> ^{-/-}	Embryonic lethal (embryonic day 10.5) Defects of normal vascular development in embryo and yolk sac Lack of aortic arch vessels	[62,63]
<i>FoxO3a</i> ^{-/-}	Viable Abnormal ovarian follicular development Hematological abnormalities Pituitary adenoma/female, ovarian stromal tumor	[62,64,65]
<i>FoxO4</i> ^{-/-}	Viable Impaired neointima formation Lung adenoma/adenocarcinoma Pituitary adenoma/female	[62,65]
<i>Mx-Cre</i> ⁺ , <i>FoxO1</i> ^{3/4} ^{L/L}	Lymphoblastic thymic lymphoma Hemangioma	[65,66]

FoxO1 is highly expressed in developing embryonic vasculature. FoxO1 has a crucial role in vascular formation [60]. Furthermore, another group demonstrated that *FoxO1*^{-/-} had defects in the branchial arches and severe cardiac failure due to the lack of aortic arch vessels and suggested that FoxO1 is indispensable for “vasculogenesis” [61]. FoxO1 is involved in the later stages of branchial arch formation from precursor cells or, alternatively, in the migration or survival of a restricted population of neural crest cells contributing to the formation of the first and second branchial arches at a specific stage.

3.2. *FoxO3* knockout mice

FoxO3a^{-/-} mice are viable and have hematological abnormalities (a mild compensated anemia with associated reticulocytosis) and a decreased rate of glucose uptake. Furthermore, *FoxO3a*^{-/-} female mice have an age-dependent reduced fertility due to abnormal ovarian follicular development. These mice showed early and widespread initiation of follicular growth and subsequently early atretic change by 9.5 weeks and noticeable absence of growing follicles [62,60]. These data suggest that FoxO3a may have a suppressive effect on initiation of follicular growth by affecting the mechanism intrinsic to the ovary. Furthermore, *FoxO3a*^{-/-} mice showed increased occurrences of ovarian stromal tumor and female pituitary adenoma [63].

3.3. *FoxO4* knockout mice

FoxO4^{-/-} mice are also viable and grossly indistinguishable from their littermate controls. Histological analyses of *FoxO4*^{-/-} mice did not identify any consistent abnormalities [60]. However, very recently, it has been reported that *FoxO4*^{-/-} mice showed increased occurrences of lung adenoma/adenocarcinoma and female pituitary adenoma [63]. These increased occurrences of pituitary adenoma in both female *FoxO3a*^{-/-} and *FoxO4*^{-/-} mice suggest to us that FoxOs might have some important physiological roles in pituitary. Indeed, FoxO1 is expressed abundantly in anterior pituitary lobe (Fig. 2).

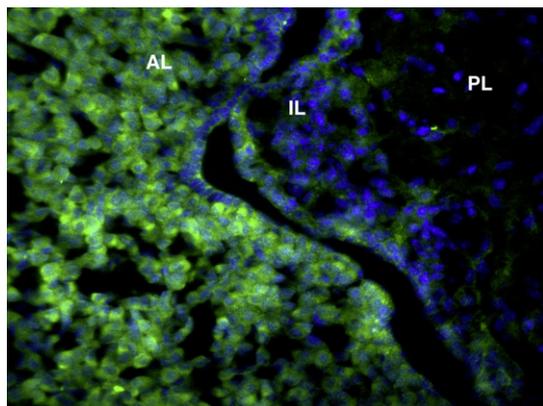


Fig. 2. FoxO1 is expressed in mouse pituitary lobes. Immunohistochemistry with anti-FOXO1 antibody (green) (ab12161, abcam) and DAPI (blue) demonstrates that FoxO1 is expressed mainly in anterior lobe of mouse pituitary and FoxO1 is localized in cytosol. AL, anterior lobe; IL, intermediate lobe; PL, posterior lobe.

3.4. *Mx-Cre*⁺; *FoxO1*3/4^{L/L}

From several studies using cell lines, FoxO proteins are involved in apoptosis or cell cycle arrest. Therefore, phenotypes of *FoxO3a*^{-/-} or *FoxO4*^{-/-}, the lack of an overt tumor-prone phenotype, were unanticipated. These studies suggested the possibility of the physical and functional relatedness and overlapping expression patterns of the FoxOs or of a relatively minor role in cancer suppression and vascular biology of FoxOs. Paik et al. generated the inducible, widespread somatic FoxO deleted adult mice, *Mx-Cre*⁺; *FoxO1*3/4^{L/L}. These mice developed aggressive CD4⁺CD8⁺ lymphoblastic thymic lymphomas, an age-progressive hamartomatous phenotype in the endothelial cell lineage with widespread hemangiomas [63]. These extensive studies suggest that tumor formation needs inactivation of all three FoxOs due to extensive functional redundancy and that FoxO1 is the most physiologically important regulator of endothelial stability, which is consistent with *FoxO1*^{-/-} mice. Furthermore, the findings that *Mx-Cre*⁺; *FoxO1*3/4^{L/L} showed abnormalities of the hematopoietic system, which were not observed in single or double knockout mutants of FoxOs, suggest the functional redundancy in hematopoietic system and a critical role for FoxOs in this system [64].

4. Metabolic effects of FoxOs in insulin-responsive tissues

For a decade, the biology of FoxOs provides a glimpse into the complex relationship among cellular proliferation, transformation, and metabolism. The researches on metabolism lead to FoxO1 as the long-sought insulin-regulated transcription factor responsible for insulin action on gene expression [5]. The identification of DAF-16 as a negative regulator of DAF-2 signaling pathway in *C. elegans* reminded us FoxOs as antagonistic effectors in insulin actions. At the present time, various kinds of target genes of FoxOs have been identified in insulin-responsive tissues, which include liver, pancreatic β -cell, fat, skeletal muscle, hypothalamus, and endothelial cells. In this paragraph, we will focus on metabolic effects of FoxOs in these insulin-responsive tissues or cells and discuss them (Fig. 3).

4.1. Liver

The hepatic metabolic effects of FoxOs in liver have been diverged into mainly two categories, gluconeogenesis and triglyceride metabolism. In mammals, at CR or food-deprivation state, hepatic glucose production is increased and glucose is supplied to brain where glucose is an only source of energy. Genes that are involved in gluconeogenesis, which include *glucose 6-phosphatase (G6pc)* and *phosphoenolpyruvate carboxykinase (Pck1)*, are upregulated at fasting, insulin deficient or insulin resistant state and downregulated at fed state or by insulin [65,66]. The promoter regions of these two genes have the insulin-responsive elements (IREs) with a T(G/A)TTT(T/G)(G/T) core sequence, which is quite similar to FoxO-responsive elements (FREs). In vitro studies, such as reporter and gel shift assays, demonstrated that *G6pc* and *Pck1* are target genes of FoxOs. However, the mechanism how FoxOs are involved in regulation of their gene expression is quite complex. Indeed, studies using transgenic mice, which overexpressed a constitutively nuclear FoxO1 in liver, enhanced *G6pc*

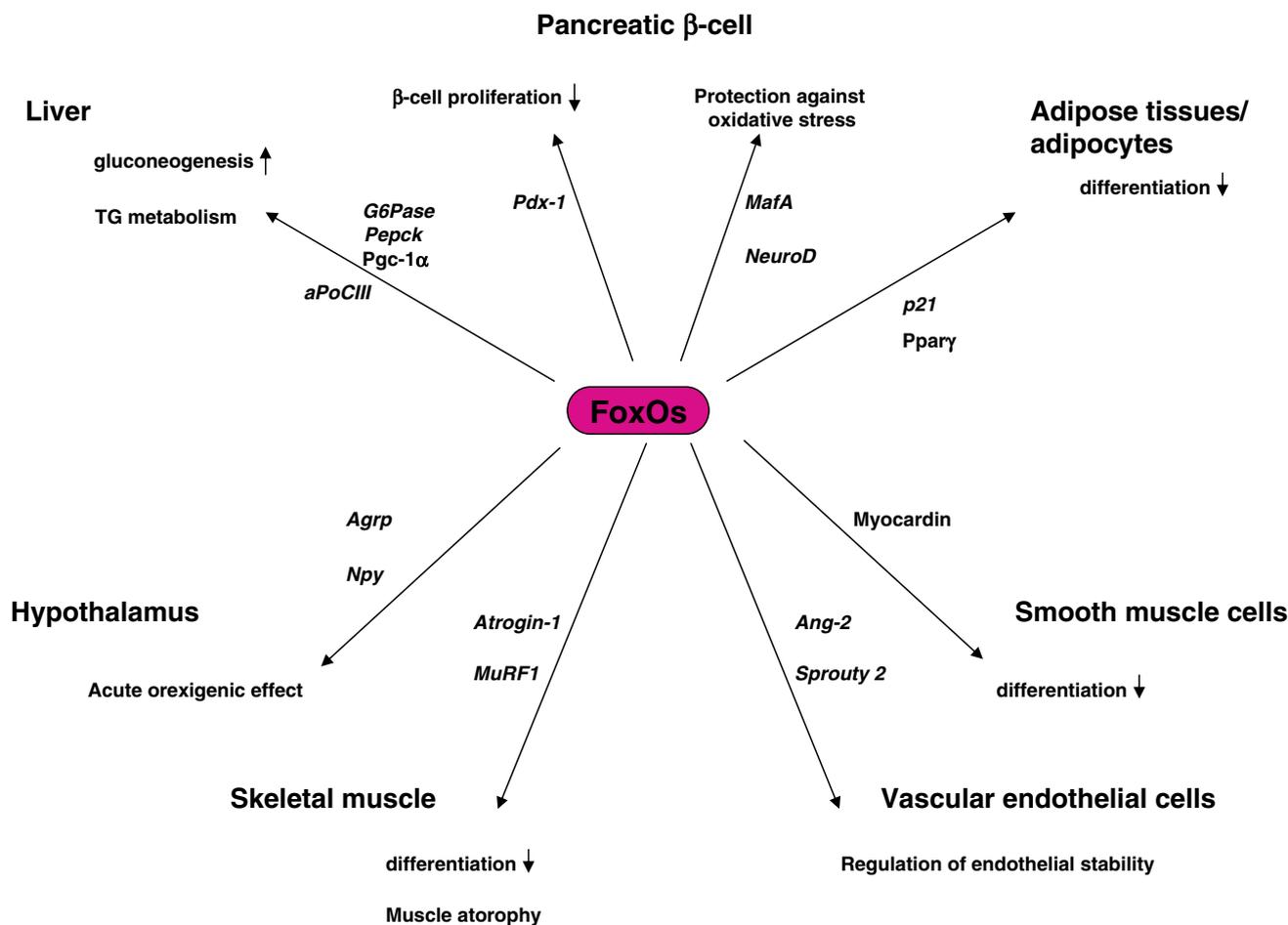


Fig. 3. The summary of metabolic effects of FoxOs in several tissues or cells. In liver, FoxO1 interacts with Pgc-1 α , induces *G6pc* and *Pck1* gene expression, and increases hepatic glucose production. Furthermore, FoxO1 induces *aPoCIII* gene expression and causes hypertriglyceridemia. In pancreatic β -cells, FoxO1 inhibits *Pdx-1* gene expression and β -cell proliferation. In contrast, FoxO1 induces *MafA* and *NeuroD* gene expression and confers protection against oxidative stress onto β -cell. In adipocytes, FoxO1 inhibits differentiation through early induction of *p21 Ppar γ* gene expression. In hypothalamus, acute overexpression of a constitutively nuclear FoxO1 induces *Agrp* or *Npy* gene expression and induces orexigenic effect. In myoblasts, FoxO1 inhibits differentiation to myotubes. In skeletal muscle, FoxOs induce *Atrogin-1* and *MuRF1* gene expression and leads to muscle atrophy. In vascular endothelial cells, FoxOs regulate endothelial stability through induction of *Ang-2* and *Sprouty 2* genes. In smooth muscle cells, FoxO4 inhibits differentiation through inhibitory interaction with myocardin protein.

but not *Pck1* gene expression [67]. Identification of FoxOs as proteins binding to IREs accounts for inhibition of basal *Igf1* gene expression by insulin and at least in part for inhibition of *G6pc* gene expression by insulin. However, regulation of *Pck1* by FoxOs remains questionable. Mutagenesis of the IRE in *Pck1* promoter region limited dexamethasone-induced *Pck1* gene expression, consistent with its proposed role as an accessory factor site. Overexpression of WT FoxO1 also limited dexamethasone induction, which could result from displacement of another more active factor. Mutagenesis of the IRE in the promoter region of *Pck1* had no effect upon insulin's inhibition of induction by PKA or by dexamethasone [68]. Furthermore, in glucogenic kidney epithelial cells, LLC-PK1-FBPase⁺ cells that have no expression of FoxO1, overexpression of FoxO1 confers insulin inhibition on Dex-cAMP-induced *G6pc* but not on *Pck1* gene expression [69]. However, the following studies support that FoxO1 may regulate *Pck1* gene expression. The decrease in nuclear FoxO1 decreases *Pck1* and *G6pc* gene expression, decreasing gluconeogenesis and reducing fasting blood glucose. This has been demon-

strated in vivo using adenoviral mediated transfer of a dominant negative FoxO1 into *Lep^{rd/db}* mice [70].

Furthermore, antisense oligonucleotides of FoxO1 inhibit *Pck1* and *G6pc* gene expression in primary hepatocytes stimulated with glucagons. Intraperitoneal administration of FoxO1 ASOs decrease *Pck1* and/or *G6pc* gene expression with reduced hepatic glucose production in lean and diet-induced obesity mice and improved glucose tolerance in both groups [71]. These findings support the previous finding that FoxO1 increases hepatic gluconeogenesis through involvement of *G6pc* and *Pck1* gene expression but suggest that FoxO1 is necessary but not sufficient for *Pck1* gene expression. Indeed, recently, it has been reported that liver-specific FoxO1 knockout mice showed 40% reduction of glucose levels at birth and 30% reduction in adult mice after 48 h fast. These findings support the previous hypothesis that FoxO1 is required for hormonal regulation of hepatic glucose production [72]. Microarray study using liver from transgenic mice overexpressing a constitutively nuclear FoxO1 demonstrated that FoxO play a role in the regulation of multiple aspects of

metabolism in liver [73]. The expression of several genes involved in promoting hepatic glucose production was increased in transgenic mice, which include *Pck1* and *G6pc*. Furthermore, *aquaporin 9*, which promotes hepatic uptake of glycerol, was increased. The expression of multiple genes promoting the catabolism of proteins and amino acid, which also provides important substrates for gluconeogenesis, was increased. Therefore, FoxOs may promote hepatic glucose production by multiple mechanisms.

Coactivator PGC-1 α controls gluconeogenic gene expression by coactivating FoxO1 and HNF-4. During periods of fasting, once glucagon activates protein kinase-A and followed by phosphorylation of CREB, *Pck1* gene is immediately upregulated. Furthermore, glucagons and glucocorticoid induce PGC-1 α protein level and subsequently up-regulates *G6pc* and *Pck1* gene expression by coactivating FoxO1 and HNF-4 [74]. PGC-1 α is induced in liver on fasting, and elevated in several models of diabetes or deficiency in insulin actions. Insulin does not have a direct effect on PGC-1 α expression in cultured hepatocytes or liver. The effect of insulin on PGC-1 α expression in vivo is probably controlled, at least in part, through counter-regulatory hormones, especially glucagon [75]. In Fao hepatocytes, overexpression of PGC-1 α increased *G6pc* and *Pck1* gene expression. In contrast, a dominant negative FoxO1 (aa1–256), which has no transactivation domain, inhibits PGC-1 α -induced gene expression of *G6pc* and *Pck1* [76]. These data suggest that PGC-1 α requires intact FoxO1 signaling to activate the gluconeogenic genes and that PGC-1 α and FoxO1 functionally interact.

The apolipoprotein apoC-III plays an important role in TG metabolism. The apolipoprotein apoC-III functions as an inhibitor of lipoprotein lipase (LPL), a key enzyme in the hydrolysis of TG in VLDL and chylomicrons. Elevated plasma apoC-III are associated with impaired hydrolysis and retarded clearance of TG-rich particles due to inhibition of hepatic lipase activity and of apoE-mediated hepatic uptake of TG-rich remnants, resulting in the accumulation of VLDL-TG and chylomicrons in plasma and the development of hypertriglyceridemia. ApoC-III is mainly produced in liver and its hepatic production is subject to insulin inhibition. FoxO1 is an effective mediator of insulin in modulating hepatic apoC-III expression and in affecting plasma TG metabolism. Transgenic mice expressing a constitutively nuclear FoxO1 allele exhibited hypertriglyceridemia. In diabetic *NOD* and *Lepr^{db/db}* mice, hepatic FoxO1 expression was deregulated, as evidenced by elevated FoxO1 production along with its increased nuclear localization in liver. These data suggested that FoxO1 deregulation associated with insulin deficiency or insulin resistance played an important role in linking impaired insulin action to aberrant apoC-III production in the pathophysiology of diabetic hypertriglyceridemia [77]. In contrast, acute overexpression of a constitutively nuclear FoxO1 affects hepatic glucose and lipid metabolism alternatively. Acute overexpression of FoxO1 mutant using adenoviral system increased hepatic TG content and reduced plasma insulin, glucose, TG and β -hydroxybutyrate levels. Increased FoxO1 activity leads to lipid accumulation by activating TG synthesis through *Srebf1* and inhibiting FFA oxidation through decreased expression of *Ppara78*. These two different results about effects of overexpression of a constitutively nuclear FoxO1 on TG metabolism might be due to positive feedback loop, interaction with

mTOR pathway or unidentified mechanisms (discussed below).

4.2. Pancreatic β -cell

Several studies using genetically mice models demonstrated that insulin/IGF-1 signaling in pancreatic β -cell has important roles in insulin secretion or β -cell proliferation. Knockout mice of insulin receptor substrate (*Irs*)-2 develop fasting hyperglycemia, followed by insulin deficiency and death from non-ketotic hyperosmolar coma due to combined insulin resistance in peripheral tissues and impaired growth of pancreatic β -cells [79,80]. β -cell specific insulin receptor (*Ir*) knockout mice resulted in progressive glucose intolerance due to impaired insulin secretion [81]. β -cell-specific *Igf-1r* knockout mice resulted in age-dependent impairment of glucose tolerance, associated with a decrease of glucose- and arginine-dependent insulin release [82,83]. Furthermore, a recent study of β -cell specific 3-phosphoinositide-dependent protein kinase 1 (*Pdk1*), which is a serine-threonine kinase that mediates signaling downstream of PI 3-kinase, knockout mice demonstrated that *Pdk1* is important in maintenance of pancreatic β -cell mass [84]. However, molecules that are located in downstream of *Ir/Igf-1* signaling pathway in pancreatic β -cell have not been known yet for a long time.

Mice models using gain-of-function or loss-of-function mutant of *FoxO1* suggested that FoxO1 had important roles in β -cell proliferation. Transgenic mice in which a constitutively nuclear FoxO1 (S253A) was expressed both in liver and pancreatic β -cell (*Tr305*) showed early onset of diabetes due to inhibition of compensatory β -cell growth in response to peripheral insulin resistance through suppression of the pancreatic transcription factor pancreas/duodenum homeobox gene-1 (*Pdx1*) expression [67]. This mutant transgene prevents β -cell replication in another 2 models of β -cell hyperplasia, 1 due to peripheral insulin resistance (Insulin receptor transgenic knockouts) and 1 due to ectopic local expression of IGF2 (Elastase-IGF2 transgenics) [85]. Haploinsufficiency for *FoxO1* reversed β -cell failure in *Irs2* knockout mice through partial restoration of β -cell proliferation and increased expression of *Pdx1* [86]. Haploinsufficiency of *FoxO1* in β -cell specific *Pdk1* knockout mice also resulted in a marked increase in the number of β -cells and resulted in the restoration of glucose homeostasis [84]. These data suggest that FoxO1 is a downstream of *Irs2/Pdk1* signaling and inhibits compensatory β -cell proliferation in insulin resistant state.

FoxO1 is also involved in another part of β -cell function through a different mechanism. Chronic exposure to elevated glucose concentrations causes a deterioration of β -cell function (glucose toxicity). This glucose toxicity is thought to arise as a consequence of chronic oxidative stress, when intracellular glucose concentrations exceed the glycolytic capacity of the β -cell. A recent study suggested a new role of FoxO1 in the protection against β -cell failure. Exposure of β TC-3 cells to hydrogen peroxide, an inducer of superoxide formation, targets FoxO1 to promyelocytic leukemia-associated protein (Pml)-containing nuclear subdomains and increases expression of the *Ins2* gene transcription factors NeuroD and MafA in a FoxO1-dependent manner. Acetylation is required to target FoxO1 to nuclear Pml bodies, while deacetylation by the NAD-dependent deacetylase Sirt1 is required to induce gene expression. Acetylation increases FoxO1 stability and targeting FoxO1 to Pml

nuclear bodies due to prevention of ubiquitin-dependent degradation, where it is deacetylated by Sirt1 to become transcriptionally active. These provide a novel mechanism linking glucose- and growth factor receptor-activated pathways to protect β -cells against oxidative damage via FoxO proteins [41].

4.3. Adipose tissues

Adipose tissue now sits on a central position of in vivo glucose metabolism. Obesity is resulted from positive balance between energy intake and energy expenditure. Excess amount of intake is stored as triglycerides in white adipose tissue [87]. Obesity leads to insulin resistance in peripheral tissues due to altered expression or secretion of adipokines and infiltration of macrophages and subsequently causes type 2 diabetes, hyperlipidemia, and hypertension and finally causes atherosclerosis [88].

FoxOs are expressed highly in both white and brown adipose tissues [33]. Their expression levels are changed by high fat diet (Nakae J., unpublished observation). Therefore, it can be speculated that FoxOs may have some important roles in these tissues. Insulin or other growth factors are important for adipocyte differentiation [89–92]. However, it has not been known about the mechanism how these growth factors can regulate adipocyte differentiation. FoxO1 is expressed little in preadipocyte cell lines but, during differentiation, FoxO1 expression level is increased gradually. At the end of clonal expansion, FoxO1 is localized in nucleus even in the presence of insulin and serum. After that, FoxO1 is distributed into cytoplasm. A constitutively nuclear FoxO1 (T24A/S253D/S316A: ADA) can inhibit adipocyte differentiation of 3T3-F442A cells due to early induction of p21. Furthermore, a dominant negative FoxO1, in which a C-terminal transactivation domain is truncated, can restore adipocyte differentiation of embryonic fibroblasts from insulin receptor knockout mice [33]. These data suggest that FoxO1 may be a linker between growth factor-signaling and transcriptional program in adipocytes during differentiation and that FoxO1's activity is finely regulated in a differentiation stage-dependent manner.

Haploinsufficiency of *FoxO1* in heterozygous insulin receptor knockout mice or high fat-dieted mice improved insulin sensitivity [67,33]. In these mice, gene expression in adipose tissues was altered compared with high fat-dieted wild type mice. These findings suggest several possibilities that (1) FoxO1 in adipose tissues may have many target genes and up- or down-regulate their expression in insulin resistant state or (2) FoxO1 can regulate expression or activity of one or two signaling molecules at transcriptional level or through protein–protein interaction and can regulate expression of genes in adipose tissues as a whole.

Dowell et al. described an antagonistic relationship between FoxO1 and Pparg where FoxO1 decreased the formation of a Pparg/Rxr/DNA complex [93]. Therefore, reducing *FoxO1* mRNA expression may lead to improvements in Pparg activity. Furthermore, it has been reported that FoxO1 can inhibit *PPAR* γ 1 and *PPAR* γ 2 promoter activity through direct interaction between FoxO1 and *PPAR* γ promoter [94]. Therefore, FoxO1 may suppress *PPAR* γ expression at the transcriptional level.

Daf-2 mutants or dauer in *C. elegans* have increased lipid droplets. Where are these lipids in case of mammalian at insu-

lin resistant state? Fat-specific insulin resistant mice (*FIRKO*) showed decreased adiposity [95]. It is speculated that lipid droplets in *C. elegans* at dauer stage may be equal to steatosis or hypertriglyceridemia in mammalian. For elucidation of the physiological roles of FoxOs in white and brown adipose tissues, we have to wait for studies using genetically modified mice, such as adipose tissue-specific knockout or transgenic mice of FoxOs.

4.4. Hypothalamus

Recent studies revealed an important role for central insulin action in glucose metabolism. The administration of insulin into third ventricular increased the ability of insulin to inhibit hepatic glucose production in rat [96] whereas central administration of insulin receptor antisense caused hepatic insulin resistance [97]. Brain-specific insulin receptor knockout mice showed mild hyperphasia and adiposity in female, diet-sensitive obesity and defects in reproductive function [98]. Furthermore, mice, which had modestly reduced insulin receptor (*Insr*) in liver and a near complete ablation of *Insr* in the arcuate and paraventricular nuclei of the hypothalamus, had marked hyperinsulinemia and developed late-onset type 2 diabetes [99]. These findings suggest that insulin signaling in CNS is important for regulation of energy homeostasis. *Irs* proteins lie downstream of the *Insr* and *Igf-1r*. Female *Irs2* knockout mice showed infertile, hyperphagic, leptin resistant and developed obesity [100,101]. Mice lacking *Irs2* in both pancreatic β -cell and hypothalamic neuronal population using a rat insulin 2 promoter Cre (*RIPCre*) recombinase or in all neuron using Nestin promoter Cre (*NesCre*) demonstrated hypothalamic dysfunction [102–104]. These suggest that *Irs2* protein has a critical role in hypothalamic function. Furthermore, a recent study demonstrated that PI3K is activated by both insulin and leptin in *Pomc* neuron, whereas in *Agrp* neurons, it is activated by insulin but inhibited by leptin [105]. Thus, the PI3K pathway in hypothalamus has been implicated in regulation of food intake and energy homeostasis. However, it has not been known about the molecular mechanism of a downstream of *Insr/Igf-1r-Irs2-PI3K* yet.

In 2006, two groups have reported about roles of FoxO1 in hypothalamus [106,107]. Both groups showed that FoxO1 is expressed in hypothalamus, which includes the arcuate nucleus, ventromedial hypothalamus and dorsomedial hypothalamus. Acute overexpression of a constitutively nuclear FoxO1, in which three Akt-mediated phosphorylation sites were substituted to alanine, in rat or mouse arcuate nucleus by a stereotactic delivery caused increased food intake, body weight, and leptin and/or insulin resistance. Furthermore, acute overexpression of a dominant negative FoxO1, in which a C-terminal transactivation domain was truncated, haploinsufficiency, or knockdown of FoxO1 decreased food intake and increased leptin sensitivity. A constitutively nuclear FoxO1 increased orexigenic neuropeptides, *Agouti-related protein (Agrp)* and *neuropeptide Y (Npy)*, gene expression, and inhibited *pro-opiomelanocortin (Pomc)* gene expression through competition with Stat3 on *Pomc* promoter region. These novel findings suggest that FoxO1 in arcuate nucleus may activate feeding behavior.

However, there are still points, which should be clarified. The first one is which neurons in hypothalamus, especially in the arcuate nucleus, are truly important for FoxO1's action

for food intake. Because these two studies used the stereotactic delivery of adenovirus encoding a constitutively nuclear FoxO1, it was not accomplished about overexpression in particular neuron-specific manner. The second point is “how about the long term effects of overexpression of FoxO1 in hypothalamus on food intake and glucose metabolism”. The last point is whether signaling molecules of upstream of FoxO1, such as Akt or Pdk1, are expressed and functioned truly in hypothalamic specific neurons, such as *Agrp/Npy* or *Pomc/Cart* neurons, or whether these molecules are co-expressed with FoxO1 in these neurons. For the purpose of these analyses, studies using neuron-specific FoxOs knockout mice or neuron-specific FoxO transgenic mice should be waited.

4.5. Skeletal muscle

FoxOs are expressed in skeletal muscle and their expression levels are changed in response to energy metabolism. Expression levels of *FoxO1* and *FoxO3a* were increased in skeletal muscle by starvation and glucocorticoid treatment [108]. Skeletal muscle has an important role for the regulation of glucose metabolism. These findings facilitate us to speculate that FoxOs may have some physiological roles in skeletal muscle.

It has been recognized that IGF-1 is involved in myoblast differentiation through PI3K/Akt [109]. Inactivation of IGF signaling by targeted mutagenesis of IGF1 receptor leads to muscle hypoplasia [110], whereas overexpression of IGF1 results in enlarged myofibers [111]. However, downstream targets of PI3K/Akt have not been known for a long time. Recent findings demonstrated that FoxOs have important roles in myoblast differentiation [112]. In C₂C₁₂ cells, FoxOs protein expression is decreased during differentiation and phosphorylated in myotubes. Overexpression of a constitutively nuclear FoxO1 in C₂C₁₂ cells inhibits differentiation from myoblasts to myotubes. In contrast, a dominant negative FoxO1 partially rescues inhibition of C₂C₁₂ differentiation mediated by wortmannin. Furthermore, knockdown of endogenous FoxOs resulted in increased myosin expression. In P19 teratocarcinoma cells, a dominant negative FoxO1 enhances myoblast differentiation and a constitutively nuclear FoxO1 inhibits it. These findings suggest that FoxOs are implicated in IGF-dependent myoblast differentiation.

On the other hand, mass and fiber size in adult skeletal muscle is regulated in response to changes in workload, activity, or pathological conditions, which include cancer, diabetes mellitus, sepsis, and Cushing's syndrome [113]. The control of muscle mass is regulated by a dynamic balance between anabolic and catabolic processes. Hypertrophy of muscle is associated with increased protein synthesis, while atrophy enhances protein degradation. Muscle atrophy is characterized by increased protein degradation processes, especially the ATP-dependent ubiquitin-proteasome proteolytic pathway, and increased transcriptional adaptation that constitute an atrophy program. Interestingly, decreased activity of IGF-1/PI3K/Akt signaling pathway can lead to muscle atrophy [114]. It has been shown that two genes are induced in muscle atrophy [115]. These genes are *MAFbx* (*muscle atrophy F box*, *Atrogin-1*) and *MuRF1* (*muscle RING finger 1*), which function to conjugate ubiquitin to protein substrates. Indeed, *MAFbx* or *MuRF1* knockout mice showed sparing of muscle mass following denervation. In C₂C₁₂ cells, the IGF/PI3K/Akt pathway inhib-

its *atrogin-1* and *MuRF1* gene expression through inactivation of FoxOs and inhibition of FoxOs reduces the induction of *atrogin-1* gene expression induced by glucocorticoids [116,117]. Furthermore, activation of FoxOs causes dramatic atrophy of myotubes and mature muscle fibers. Indeed, during atrophy, *FoxO1* and *FoxO3a* gene expression levels are increased and reduced Akt activity causes dephosphorylation of FoxOs and activates them. These data suggest that IGF-1/PI3K/Akt signaling pathway can not only induce muscle hypertrophy through activation of protein synthesis but also inhibit the activation of muscle atrophy pathway through inhibition of FoxOs activity. A skeletal muscle-specific FoxO1 transgenic mice using human skeletal muscle α -actin promoter showed reduced body weight due to a decreased lean body mass [118]. The skeletal muscles from transgenic mice were smaller in size and dry mass, as well as paler in color than those of control mice. Furthermore, the transgenic mice showed poor glycemic control and low capacity for physical exercise. These phenotypes of the transgenic mice are consistent with the findings of cell lines described above and support the previous findings that FoxO1 negatively regulates skeletal muscle mass.

4.6. Vascular endothelial cells

Insulin receptors (IRs) have been demonstrated on endothelial cells of both large and small blood vessels [119]. The findings that vascular endothelial cell-specific IR knockout mice (*VENIRKO*) on a low-salt diet showed significant insulin resistance suggest that insulin signaling in endothelial cells participates in the mechanism that correct whole-body insulin sensitivity and vascular function [120].

The previous finding that *FoxO1*-null mice showed defects in vasculogenesis [60,61] and also the recent report that inducible, wide spread somatic FoxO deleted adult mice, *Mx-Cre⁺; FoxO1/3/4^{LL}*, caused systemic occurrence of haemangioma in limited tissues, which include uterus, liver, and muscle [63]. These data suggest a physiological role of FoxOs in vascular system and that FoxO1 is the most physiologically important factor of endothelial stability. *Sprouty 2* was identified as a target molecule of FoxOs. Because *Sprouty 2* is an inhibitor of growth factor receptor tyrosine kinase signaling, FoxOs might regulate EC response to extracellular angiogenic stimuli [63].

It has been reported that angiopoietin (Ang)-1, a regulator of vascular maturation and stability as well as an endothelial cell survival factor, inhibits FoxO1 activity by phosphorylation through Tie2/Akt pathway [121]. FoxO1 modulates expression of genes, which are associated with blood vessel destabilization and remodeling and apoptosis. FoxO1 induces *Ang-2* gene expression. Ang-2 is an Ang-1 antagonist and binds but does not activate Tie2. Ang-2 is associated with blood vessel destabilization and remodeling. The positive feedback that FoxO1 activates FoxO1 itself through antagonizing Ang-1/Akt pathway through induction of Ang-2 may explain that FoxO1 can change expression of various genes that are involved in vessel destabilization, matrix remodeling in vasculature, endothelial cell migration, or tube formation. The alteration of gene expression in blood vessel suggests that FoxO1 activation is likely to be coupled to changes in the interaction between endothelial cells and the surrounding support cells and matrix.

4.7. Vascular smooth muscle cells

Abnormal proliferation and migration of smooth muscle cells (SMCs) contribute to the pathogenesis of atherosclerosis, restenosis following angioplasty, hypertension, and leiomyosarcoma [122]. SMCs do not differentiate terminally and can transit between a quiescent, contractile phenotype and a proliferative synthetic phenotype in response to physiological and pathological stimuli [123]. In response to vascular injury, differentiated SMCs proliferate and dedifferentiate. At the same time, SMCs express contractile proteins required for normal cardiovascular homeostasis. Expression of genes encoding smooth muscle-specific contractile proteins and extracellular matrix proteins is characteristic for vascular SMC differentiation and phenotypic modulation.

A CarG box (CC(A/T)₆GG), which is the binding site in the promoter region for serum response factor (SRF), a MADS (MCM1, Agamous, Deficiens, SRF) box transcription factor, is important for expression of SMC contractile genes [124]. Myocardin is a potent coactivator of SRF that can activate the program of SM differentiation in transfected fibroblasts. Myocardin is sufficient and necessary for activation of SM contractile protein genes [125]. Activation of the PI3K/Akt signaling pathway promotes SMC differentiation and FoxO4 is the most abundant family member in myocyte-containing tissues. Liu, et al. demonstrated that a consti-

tutively nuclear FoxO4 inhibits SMC differentiation through a mechanism independent of DNA binding, whereas knock-down of endogenous FoxO4 in SMCs increased expression of SM contractile genes. They also showed that FoxO4 forms a ternary complex with myocardin and SRF and inhibit the transcriptional and myogenic activities of myocardin [126]. Furthermore, it has been reported that FoxO4 is also a transcriptional activator of *myocardin* [127]. Therefore, FoxO4 might fine-tune the potent transcriptional activity of myocardin and regulate the differentiation of SMCs. Indeed, the finding that *FoxO4*-null mice display impaired neointima formation after carotid artery ligation, which is a potent stimulus for phenotypic modulation of SMCs, suggests that FoxO4 is required for vascular remodeling following injury to the vessel wall.

5. FoxOs can regulate activity of insulin signaling molecules

FoxOs cause insulin resistance through induction of expression of several genes, which are involved in metabolism. However, recent findings suggest that FoxOs can alleviate insulin resistance through positive feedback loop on insulin signaling (Fig. 4) and also that FoxOs can interact with mTOR pathway by several ways.

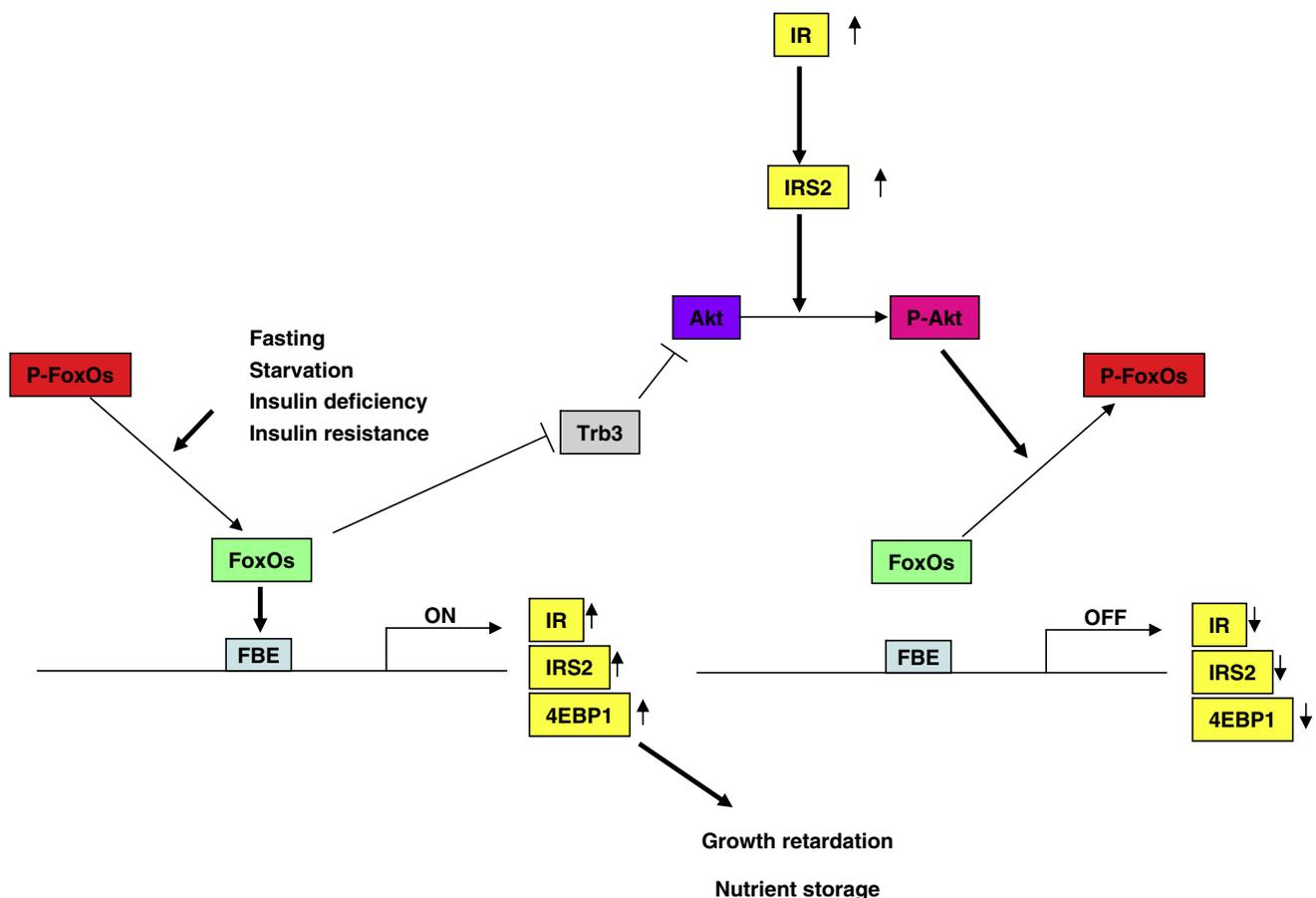


Fig. 4. Positive feedback loop of FoxOs on insulin signaling. Fasting, starvation, insulin deficiency, or insulin resistance causes dephosphorylation of FoxOs and nuclear import. Nuclear FoxOs induce expression of their target genes, which include insulin receptor (IR), insulin receptor substrate 2 (IRS2) and 4EBP1. Enhanced expression of IR and IRS2 phosphorylate and activate Akt, leading to phosphorylate and export FoxOs nucleus again. Increased expression of 4EBP1 causes growth retardation and sparing of nutrient storage.

5.1. Positive feedback loop on insulin signaling

FoxOs has inhibitory effects on insulin actions, such as increased glucose production in liver, inhibition of compensatory pancreatic β -cell proliferation in response to peripheral insulin resistant state, or increased food intake through regulation of gene expression of hypothalamic neuropeptides. However, FoxOs provide rapid changes on insulin signaling in response to a nutritional status or hormonal environment between fasting and fed state.

In *Drosophila*, *Drosophila* FoxO (dfoxo) activates transcription of the insulin receptor (*dir*) and translational repressor *d4ebp* expression [128]. In the nutrition-limited state, dfoxo enhances *dir* expression to prime for changes in nutrient availability and *4ebp* expression to inhibit growth in order to spare nutrient storage. FoxO1 also increases one of insulin receptor substrate (*Irs*), *Irs2*, at transcriptional level [129]. *Irs2* has several important roles in metabolism in a tissue-specific manner. The induction of *Irs2* by activated FoxO1 in insulin resistant state may improve insulin sensitivity. Furthermore, recent reports demonstrated that a constitutively nuclear FoxO1 (ADA) increased Akt phosphorylation in Trb3 (Tribbles homolog 3)-dependent or -independent manner [78,130].

Many signaling pathways are converged on FoxO1 and FoxO1 not only induces or represses expression of target genes, which are involved in metabolism in each insulin-responsive tissue, but also performs transcriptional control on signaling molecules. These novel findings make FoxO1 a central metabolic regulator in glucose and lipid metabolism.

5.2. Interaction between FoxOs and mTOR pathway

It has been reported that ribosomal p70 S6 kinase (p70S6K) enhances IRS-1 serine phosphorylation, which lead to decreased Akt phosphorylation and causes insulin resistance. Therefore, mTOR/p70S6K signaling pathway can modulate IRS-1/PI3k/Akt/FoxO pathway. The activity of mTOR/p70S6K pathway is regulated by TSC2 (also known as tuberlin). TSC1 and TSC2 physically associate in vivo and form a heterodimeric complex. Akt-phosphorylation of TSC2 leads to the functional inactivation of the TSC1–TSC2 complex and results in mTOR activation leading to phosphorylation of two main mTOR substrates, p70S6K and eukaryotic initiation factor 4E-binding protein (4E-BP1), and elevated mRNA translation.

In *Drosophila*, one of hypomorphic mutation of dTOR, a dTOR P-element insertion (*dTOR^{71P}* mutant), showed lipid breakdown due to increased lipase activity and increased conversion of lipids to ketone bodies, decreased blood glucose levels due to increased production of insulin-like peptide (DILP2) in insulin producing cells (IPCs). In contrast, systemic expression of a constitutively nuclear dFOXO (dFOXO-TM) results in increased lipid levels, decreased *DILP2* mRNA levels, and high glucose levels. Interestingly, reduced dTOR activity in *dTOR^{71P}* can block dFOXO-TM-mediated relevant metabolic targets to reverse the increased glucose and lipid phenotype. Therefore, the dTOR mutant phenotype can block activated dFOXO-TM-mediated insulin resistance phenotypes although the mechanism by which reduced dTOR activity inhibits activity of a constitutively nuclear dFOXO (dFOXO-TM) has not been clear yet [131].

Recently, Melted, which is associated with both dTsc1 and dFOXO in *Drosophila*, was identified as an activator of

dTOR signaling and an inhibitor of dFOXO. The melted gene encodes a C-terminal Pleckstrin Homology (PH) domain protein and can recruit the Tsc1-Tsc2 complex to the plasma membrane and modulate its output via dTOR pathway and can also recruit dFOXO to the membrane in an insulin-responsive manner and thereby influence expression of dFOXO's target genes. The predicted Melted protein is conserved beyond different species, *C. elegans*, mice, and human. The findings that melted mutant demonstrated 10% reduced body size and 60% of triglyceride content of wild type fly suggest that Melted activity is required in adipose tissue. Melted acts by regulating FOXO activity to control expression of genes important in fat metabolism [132]. From studies on Melted in *Drosophila*, it can be speculated that mammalian homolog of *Melted* may have some important roles in metabolism affecting both mTOR and FoxOs activity. We also identified TSC2 as a FoxO1-binding protein by yeast two hybrid screening using murine islets cDNA. Physical association between TSC2 and FoxO1 inhibits TSC2 function for inhibition to mTOR and leads to enhance p70S6K phosphorylation. The finding that knockdown of endogenous FOXO1 in HUVEC inhibits p70S6K phosphorylation suggests that FOXO1 has a physiological role in regulation of activity of mTORC1 signaling [133].

6. Conclusion

CR or starvation lowers blood glucose and insulin levels, decreases insulin actions and results in nuclear accumulation of FoxOs. FoxOs can accelerate glucose production, reduced insulin secretion, decreased fat and muscle masses and leads to spare energy in vivo. FoxOs should be necessary for survival in lower organisms evolutionally and be conserved beyond species. However, in human, excessive calorie intake causes obesity and insulin resistance. At these circumstances, FoxOs should be active even not in CR state. Deregulated and unnecessary FoxOs' activity may cause and accelerate insulin resistance and finally lead to type 2 diabetes and hyperlipidemia. The inhibition of this vicious cycle by inactivation of FoxOs may shed light on the treatment of type 2 diabetes and also the metabolic syndrome.

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References

- [1] Klass, M. and Hirsh, D. (1976) Non-ageing developmental variant of *Caenorhabditis elegans*. *Nature* 260, 523–525.
- [2] Riddle, D.L., Swanson, M.M. and Albert, P.S. (1981) Interacting genes in nematode dauer larva formation. *Nature* 290, 668–671.
- [3] Ogg, S., Paradis, S., Gottlieb, S., Patterson, G.I., Lee, L., Tissenbaum, H.A. and Ruvkun, G. (1997) The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. *Nature* 389, 994–999.
- [4] Lin, K., Dorman, J.B., Rodan, A. and Kenyon, C. (1997) daf-16: An HNF-3/forkhead family member that can function to double the life-span of *Caenorhabditis elegans*. *Science* 278, 1319–1322.

- [5] Accili, D. and Arden, K.C. (2004) FoxOs at the crossroads of cellular metabolism, differentiation, and transformation. *Cell* 117, 421–426.
- [6] Greer, E.L. and Brunet, A. (2005) FOXO transcription factors at the interface between longevity and tumor suppression. *Oncogene* 24, 7410–7425.
- [7] Clark, K.L., Halay, E.D., Lai, E. and Burley, S.K. (1993) Co-crystal structure of the HNF-3/fork head DNA-recognition motif resembles histone H5. *Nature* 364, 412–420.
- [8] Kaestner, K.H., Knochel, W. and Martinez, D.E. (2000) Unified nomenclature for the winged helix/forkhead transcription factors. *Genes Dev.* 14, 142–146.
- [9] Cantley, L.C. (2002) The phosphoinositide 3-kinase pathway. *Science* 296, 1655–1657.
- [10] Brunet, A., Bonni, A., Zigmond, M.J., Lin, M.Z., Juo, P., Hu, L.S., Anderson, M.J., Arden, K.C., Blenis, J. and Greenberg, M.E. (1999) Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* 96, 857–868.
- [11] Kops, G.J. and Burgering, B.M. (1999) Forkhead transcription factors: new insights into protein kinase B (c-akt) signaling. *J. Mol. Med.* 77, 656–665.
- [12] Nakae, J., Park, B.C. and Accili, D. (1999) Insulin stimulates phosphorylation of the forkhead transcription factor FKHR on serine 253 through a Wortmannin-sensitive pathway. *J. Biol. Chem.* 274, 15982–15985.
- [13] Rena, G., Guo, S., Cichy, S.C., Unterman, T.G. and Cohen, P. (1999) Phosphorylation of the transcription factor forkhead family member FKHR by protein kinase B. *J. Biol. Chem.* 274, 17179–17183.
- [14] Tang, E.D., Nunez, G., Barr, F.G. and Guan, K.L. (1999) Negative regulation of the forkhead transcription factor FKHR by Akt. *J. Biol. Chem.* 274, 16741–16746.
- [15] Biggs 3rd, W.H., Meisenhelder, J., Hunter, T., Cavenee, W.K. and Arden, K.C. (1999) Protein kinase B/Akt-mediated phosphorylation promotes nuclear exclusion of the winged helix transcription factor FKHR1. *Proc. Natl. Acad. Sci. USA* 96, 7421–7426.
- [16] Brunet, A., Kanai, F., Stehn, J., Xu, J., Sarbassova, D., Frangioni, J.V., Dalal, S.N., DeCaprio, J.A., Greenberg, M.E. and Yaffe, M.B. (2002) 14-3-3 transits to the nucleus and participates in dynamic nucleocytoplasmic transport. *J. Cell Biol.* 156, 817–828.
- [17] Cahill, C.M., Tzivion, G., Nasrin, N., Ogg, S., Dore, J., Ruvkun, G. and Alexander-Bridges, M. (2001) Phosphatidylinositol 3-kinase signaling inhibits DAF-16 DNA binding and function via 14-3-3-dependent and 14-3-3-independent pathways. *J. Biol. Chem.* 276, 13402–13410.
- [18] Brownawell, A.M., Kops, G.J., Macara, I.G. and Burgering, B.M. (2001) Inhibition of nuclear import by protein kinase B (Akt) regulates the subcellular distribution and activity of the forkhead transcription factor AFX. *Mol. Cell Biol.* 21, 3534–3546.
- [19] Rena, G., Prescott, A.R., Guo, S., Cohen, P. and Unterman, T.G. (2001) Roles of the forkhead in rhabdomyosarcoma (FKHR) phosphorylation sites in regulating 14-3-3 binding, transactivation and nuclear targeting. *Biochem. J.* 354, 605–612.
- [20] Jacobs, F.M., van der Heide, L.P., Wijchers, P.J., Burbach, J.P., Hoekman, M.F. and Smidt, M.P. (2003) FoxO6, a novel member of the FoxO class of transcription factors with distinct shuttling dynamics. *J. Biol. Chem.* 278, 35959–35967.
- [21] Lin, K., Hsin, H., Libina, N. and Kenyon, C. (2001) Regulation of the *Caenorhabditis elegans* longevity protein DAF-16 by insulin/IGF-1 and germline signaling. *Nat. Genet.* 28, 139–145.
- [22] Rena, G., Bain, J., Elliott, M. and Cohen, P. (2004) D4476, a cell-permeant inhibitor of CK1, suppresses the site-specific phosphorylation and nuclear exclusion of FOXO1a. *EMBO Rep.* 5, 60–65.
- [23] Woods, Y.L., Rena, G., Morrice, N., Barthel, A., Becker, W., Guo, S., Unterman, T.G. and Cohen, P. (2001) The kinase DYRK1A phosphorylates the transcription factor FKHR at Ser329 in vitro, a novel in vivo phosphorylation site. *Biochem. J.* 355, 597–607.
- [24] Oh, S.W., Mukhopadhyay, A., Svrzikapa, N., Jiang, F., Davis, R.J. and Tissenbaum, H.A. (2005) JNK regulates lifespan in *Caenorhabditis elegans* by modulating nuclear translocation of forkhead transcription factor/DAF-16. *Proc. Natl. Acad. Sci. USA* 102, 4494–4499.
- [25] Wang, M.C., Bohmann, D. and Jasper, H. (2005) JNK extends life span and limits growth by antagonizing cellular and organism-wide responses to insulin signaling. *Cell* 121, 115–125.
- [26] Essers, M.A., Weijzen, S., de Vries-Smits, A.M., Saarloos, I., de Ruiter, N.D., Bos, J.L. and Burgering, B.M. (2004) FOXO transcription factor activation by oxidative stress mediated by the small GTPase Ral and JNK. *EMBO J.* 23, 4802–4812.
- [27] Eisenmann, D.M., Maloof, J.N., Simske, J.S., Kenyon, C. and Kim, S.K. (1998) The beta-catenin homolog BAR-1 and LET-60 Ras coordinately regulate the Hox gene *lin-39* during *Caenorhabditis elegans* vulval development. *Development* 125, 3667–3680.
- [28] Logan, C.Y. and Nusse, R. (2004) The Wnt signaling pathway in development and disease. *Annu. Rev. Cell Dev. Biol.* 20, 781–810.
- [29] Reya, T. and Clevers, H. (2005) Wnt signalling in stem cells and cancer. *Nature* 434, 843–850.
- [30] Essers, M.A., de Vries-Smits, L.M., Barker, N., Polderman, P.E., Burgering, B.M. and Korswagen, H.C. (2005) Functional interaction between beta-catenin and FOXO in oxidative stress signaling. *Science* 308, 1181–1184.
- [31] Ross, S.E., Hemati, N., Longo, K.A., Bennett, C.N., Lucas, P.C., Erickson, R.L. and MacDougald, O.A. (2000) Inhibition of adipogenesis by Wnt signaling. *Science* 289, 950–953.
- [32] Tseng, Y.H., Butte, A.J., Kokkotou, E., Yechoor, V.K., Taniguchi, C.M., Kriaucinas, K.M., Cypess, A.M., Niinobe, M., Yoshikawa, K., Patti, M.E. and Kahn, C.R. (2005) Prediction of preadipocyte differentiation by gene expression reveals role of insulin receptor substrates and neclin. *Nat. Cell Biol.* 7, 601–611.
- [33] Nakae, J., Kitamura, T., Kitamura, Y., Biggs 3rd, W.H., Arden, K.C. and Accili, D. (2003) The forkhead transcription factor Foxo1 regulates adipocyte differentiation. *Dev. Cell* 4, 119–129.
- [34] Koubova, J. and Guarente, L. (2003) How does calorie restriction work? *Genes Dev.* 17, 313–321.
- [35] Imai, S., Armstrong, C.M., Kaerberlein, M. and Guarente, L. (2000) Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* 403, 795–800.
- [36] Smith, J.S., Brachmann, C.B., Celic, I., Kenna, M.A., Muhammad, S., Starai, V.J., Avalos, J.L., Escalante-Semerena, J.C., Grubmeyer, C., Wolberger, C. and Boeke, J.D. (2000) A phylogenetically conserved NAD⁺-dependent protein deacetylase activity in the Sir2 protein family. *Proc. Natl. Acad. Sci. USA* 97, 6658–6663.
- [37] Tissenbaum, H.A. and Guarente, L. (2001) Increased dosage of a sir-2 gene extends lifespan in *Caenorhabditis elegans*. *Nature* 410, 227–230.
- [38] Picard, F., Kurtev, M., Chung, N., Topark-Ngarm, A., Senawong, T., Machado De Oliveira, R., Leid, M., McBurney, M.W. and Guarente, L. (2004) Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. *Nature* 429, 771–776.
- [39] Bordone, L., Motta, M.C., Picard, F., Robinson, A., Jhala, U.S., Apfeld, J., McDonagh, T., Lemieux, M., McBurney, M., Szilvasi, A., Easlon, E.J., Lin, S.J. and Guarente, L. (2006) Sirt1 regulates insulin secretion by repressing UCP2 in pancreatic beta cells. *PLoS Biol.* 4, e31.
- [40] Moynihan, K.A., Grimm, A.A., Plueger, M.M., Bernal-Mizrachi, E., Ford, E., Cras-Meneur, C., Permutt, M.A. and Imai, S. (2005) Increased dosage of mammalian Sir2 in pancreatic beta cells enhances glucose-stimulated insulin secretion in mice. *Cell Metab.* 2, 105–117.
- [41] Kitamura, Y.I., Kitamura, T., Kruse, J.P., Raum, J.C., Stein, R., Gu, W. and Accili, D. (2005) FoxO1 protects against pancreatic beta cell failure through NeuroD and MafA induction. *Cell Metab.* 2, 153–163.
- [42] Rodgers, J.T., Lerin, C., Haas, W., Gygi, S.P., Spiegelman, B.M. and Puigserver, P. (2005) Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. *Nature* 434, 113–118.
- [43] Frescas, D., Valenti, L. and Accili, D. (2005) Nuclear trapping of the forkhead transcription factor FoxO1 via Sirt-dependent deacetylation promotes expression of glucogenic genes. *J. Biol. Chem.* 280, 20589–20595.

- [44] Nasrin, N., Ogg, S., Cahill, C.M., Biggs, W., Nui, S., Dore, J., Calvo, D., Shi, Y., Ruvkun, G. and Alexander-Bridges, M.C. (2000) DAF-16 recruits the CREB-binding protein coactivator complex to the insulin-like growth factor binding protein 1 promoter in HepG2 cells. *Proc. Natl. Acad. Sci. USA* 97, 10412–10417.
- [45] Fukuoka, M., Daitoku, H., Hatta, M., Matsuzaki, H., Umemura, S. and Fukamizu, A. (2003) Negative regulation of forkhead transcription factor AFX (Foxo4) by CBP-induced acetylation. *Int. J. Mol. Med.* 12, 503–508.
- [46] van der Horst, A., Tertoolen, L.G., de Vries-Smits, L.M., Frye, R.A., Medema, R.H. and Burgering, B.M. (2004) FOXO4 is acetylated upon peroxide stress and deacetylated by the longevity protein hSir2(SIRT1). *J. Biol. Chem.* 279, 28873–28879.
- [47] Brunet, A., Sweeney, L.B., Sturgill, J.F., Chua, K.F., Greer, P.L., Lin, Y., Tran, H., Ross, S.E., Mostoslavsky, R., Cohen, H.Y., Hu, L.S., Cheng, H.L., Jedrychowski, M.P., Gygi, S.P., Sinclair, D.A., Alt, F.W. and Greenberg, M.E. (2004) Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science* 303, 2011–2015.
- [48] Motta, M.C., Divecha, N., Lemieux, M., Kamel, C., Chen, D., Gu, W., Bultsma, Y., McBurney, M. and Guarente, L. (2004) Mammalian SIRT1 represses forkhead transcription factors. *Cell* 116, 551–563.
- [49] Daitoku, H., Hatta, M., Matsuzaki, H., Aratani, S., Ohshima, T., Miyagishi, M., Nakajima, T. and Fukamizu, A. (2004) Silent information regulator 2 potentiates Foxo1-mediated transcription through its deacetylase activity. *Proc. Natl. Acad. Sci. USA* 101, 10042–10047.
- [50] Matsuzaki, H., Daitoku, H., Hatta, M., Aoyama, H., Yoshimochi, K. and Fukamizu, A. (2005) Acetylation of Foxo1 alters its DNA-binding ability and sensitivity to phosphorylation. *Proc. Natl. Acad. Sci. USA* 102, 11278–11283.
- [51] Nakae, J., Cao, Y., Daitoku, H., Fukamizu, A., Ogawa, W., Yano, Y. and Hayashi, Y. (2006) The LXXLL motif of murine forkhead transcription factor FoxO1 mediates Sirt1-dependent transcriptional activity. *J. Clin. Invest.* 116, 2473–2483.
- [52] Matsuzaki, H., Daitoku, H., Hatta, M., Tanaka, K. and Fukamizu, A. (2003) Insulin-induced phosphorylation of FKHR (Foxo1) targets to proteasomal degradation. *Proc. Natl. Acad. Sci. USA* 100, 11285–11290.
- [53] Aoki, M., Jiang, H. and Vogt, P.K. (2004) Proteasomal degradation of the FoxO1 transcriptional regulator in cells transformed by the P3k and Akt oncoproteins. *Proc. Natl. Acad. Sci. USA* 101, 13613–13617.
- [54] Carrano, A.C., Eytan, E., Hershko, A. and Pagano, M. (1999) SKP2 is required for ubiquitin-mediated degradation of the CDK inhibitor p27. *Nat. Cell Biol.* 1, 193–199.
- [55] Tedesco, D., Lukas, J. and Reed, S.I. (2002) The pRb-related protein p130 is regulated by phosphorylation-dependent proteolysis via the protein-ubiquitin ligase SCF(Skp2). *Genes Dev.* 16, 2946–2957.
- [56] Kamura, T., Hara, T., Kotoshiba, S., Yada, M., Ishida, N., Imaki, H., Hatakeyama, S., Nakayama, K. and Nakayama, K.I. (2003) Degradation of p57Kip2 mediated by SCFSkp2-dependent ubiquitylation. *Proc. Natl. Acad. Sci. USA* 100, 10231–10236.
- [57] Bai, C., Sen, P., Hofmann, K., Ma, L., Goebel, M., Harper, J.W. and Elledge, S.J. (1996) SKP1 connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box. *Cell* 86, 263–274.
- [58] Huang, H., Regan, K.M., Wang, F., Wang, D., Smith, D.I., van Deursen, J.M. and Tindall, D.J. (2005) Skp2 inhibits FOXO1 in tumor suppression through ubiquitin-mediated degradation. *Proc. Natl. Acad. Sci. USA* 102, 1649–1654.
- [59] Sakai, T., Sakaue, H., Nakamura, T., Okada, M., Matsuki, Y., Watanabe, E., Hiramatsu, R., Nakayama, K., Nakayama, K.I. and Kasuga, M. (2007) Skp2 controls adipocyte proliferation during the development of obesity. *J. Biol. Chem.* 282, 20381–20386.
- [60] Hosaka, T., Biggs 3rd, W.H., Tieu, D., Boyer, A.D., Varki, N.M., Cavenee, W.K. and Arden, K.C. (2004) Disruption of forkhead transcription factor (FOXO) family members in mice reveals their functional diversification. *Proc. Natl. Acad. Sci. USA* 101, 2975–2980.
- [61] Furuyama, T., Kitayama, K., Shimoda, Y., Ogawa, M., Sone, K., Yoshida-Araki, K., Hisatsune, H., Nishikawa, S., Nakayama, K., Ikeda, K., Motoyama, N. and Mori, N. (2004) Abnormal angiogenesis in Foxo1 (Fkhr)-deficient mice. *J. Biol. Chem.* 279, 34741–34749.
- [62] Castrillon, D.H., Miao, L., Kollipara, R., Horner, J.W. and DePinho, R.A. (2003) Suppression of ovarian follicle activation in mice by the transcription factor Foxo3a. *Science* 301, 215–218.
- [63] Paik, J.H., Kollipara, R., Chu, G., Ji, H., Xiao, Y., Ding, Z., Miao, L., Tothova, Z., Horner, J.W., Carrasco, D.R., Jiang, S., Gilliland, D.G., Chin, L., Wong, W.H., Castrillon, D.H. and Depinho, R.A. (2007) FoxOs are lineage-restricted redundant tumor suppressors and regulate endothelial cell homeostasis. *Cell* 128, 309–323.
- [64] Tothova, Z., Kollipara, R., Huntly, B.J., Lee, B.H., Castrillon, D.H., Cullen, D.E., McDowell, E.P., Lazo-Kallanian, S., Williams, I.R., Sears, C., Armstrong, S.A., Passegue, E., Depinho, R.A. and Gilliland, D.G. (2007) FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. *Cell* 128, 325–339.
- [65] O'Brien, R.M. and Granner, D.K. (1996) Regulation of gene expression by insulin. *Physiol. Rev.* 76, 1109–1161.
- [66] O'Brien, R.M., Streeper, R.S., Ayala, J.E., Stadelmaier, B.T. and Hornbuckle, L.A. (2001) Insulin-regulated gene expression. *Biochem. Soc. Trans.* 29, 552–558.
- [67] Nakae, J., Biggs, W.H., Kitamura, T., Cavenee, W.K., Wright, C.V., Arden, K.C. and Accili, D. (2002) Regulation of insulin action and pancreatic beta-cell function by mutated alleles of the gene encoding forkhead transcription factor Foxo1. *Nat. Genet.* 32, 245–253.
- [68] Quinn, P.G. and Yeagley, D. (2005) Insulin regulation of PEPCK gene expression: a model for rapid and reversible modulation. *Curr. Drug Targets Immune Endocr. Metabol. Disord.* 5, 423–437.
- [69] Nakae, J., Kitamura, T., Silver, D.L. and Accili, D. (2001) The forkhead transcription factor Foxo1 (Fkhr) confers insulin sensitivity onto glucose-6-phosphatase expression. *J. Clin. Invest.* 108, 1359–1367.
- [70] Altomonte, J., Richter, A., Harbaran, S., Suriawinata, J., Nakae, J., Thung, S.N., Meseck, M., Accili, D. and Dong, H. (2003) Inhibition of Foxo1 function is associated with improved fasting glycemia in diabetic mice. *Am. J. Physiol. Endocrinol. Metab.* 285, E718–E728.
- [71] Samuel, V.T., Choi, C.S., Phillips, T.G., Romanelli, A.J., Geisler, J.G., Bhanot, S., McKay, R., Monia, B., Shutter, J.R., Lindberg, R.A., Shulman, G.I. and Veniant, M.M. (2006) Targeting foxo1 in mice using antisense oligonucleotide improves hepatic and peripheral insulin action. *Diabetes* 55, 2042–2050.
- [72] Matsumoto, M., Poci, A., Rossetti, L., Depinho, R.A. and Accili, D. (2007) Impaired regulation of hepatic glucose production in mice lacking the forkhead transcription factor foxo1 in liver. *Cell Metab.* 6, 208–216.
- [73] Zhang, W., Patil, S., Chauhan, B., Guo, S., Powell, D.R., Le, J., Klotsas, A., Matika, R., Xiao, X., Franks, R., Heidenreich, K.A., Sajjan, M.P., Farese, R.V., Stolz, D.B., Tso, P., Koo, S.H., Montminy, M. and Untermyer, T.G. (2006) FoxO1 regulates multiple metabolic pathways in the liver: effects on gluconeogenic, glycolytic, and lipogenic gene expression. *J. Biol. Chem.* 281, 10105–10117.
- [74] Herzig, S., Long, F., Jhala, U.S., Hedrick, S., Quinn, R., Bauer, A., Rudolph, D., Schutz, G., Yoon, C., Puigserver, P., Spiegelman, B. and Montminy, M. (2001) CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. *Nature* 413, 179–183.
- [75] Yoon, J.C., Puigserver, P., Chen, G., Donovan, J., Wu, Z., Rhee, J., Adelmant, G., Stafford, J., Kahn, C.R., Granner, D.K., Newgard, C.B. and Spiegelman, B.M. (2001) Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. *Nature* 413, 131–138.
- [76] Puigserver, P., Rhee, J., Donovan, J., Walkey, C.J., Yoon, J.C., Oriente, F., Kitamura, Y., Altomonte, J., Dong, H., Accili, D. and Spiegelman, B.M. (2003) Insulin-regulated hepatic gluconeogenesis through FOXO1–PGC-1 α interaction. *Nature* 423, 550–555.

- [77] Altomonte, J., Cong, L., Harbaran, S., Richter, A., Xu, J., Meseck, M. and Dong, H.H. (2004) Foxo1 mediates insulin action on apoC-III and triglyceride metabolism. *J. Clin. Invest.* 114, 1493–1503.
- [78] Matsumoto, M., Han, S., Kitamura, T. and Accili, D. (2006) Dual role of transcription factor FoxO1 in controlling hepatic insulin sensitivity and lipid metabolism. *J. Clin. Invest.* 116, 2464–2472.
- [79] Withers, D.J., Gutierrez, J.S., Towery, H., Burks, D.J., Ren, J.M., Previs, S., Zhang, Y., Bernal, D., Pons, S., Shulman, G.I., Bonner-Weir, S. and White, M.F. (1998) Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* 391, 900–904.
- [80] Kubota, N., Tobe, K., Terauchi, Y., Eto, K., Yamauchi, T., Suzuki, R., Tsubamoto, Y., Komeda, K., Nakano, R., Miki, H., Satoh, S., Sekihara, H., Sciacchitano, S., Lesniak, M., Aizawa, S., Nagai, R., Kimura, S., Akanuma, Y., Taylor, S.I. and Kadowaki, T. (2000) Disruption of insulin receptor substrate 2 causes type 2 diabetes because of liver insulin resistance and lack of compensatory beta-cell hyperplasia. *Diabetes* 49, 1880–1889.
- [81] Kulkarni, R.N., Bruning, J.C., Winnay, J.N., Postic, C., Magnuson, M.A. and Kahn, C.R. (1999) Tissue-specific knockout of the insulin receptor in pancreatic beta cells creates an insulin secretory defect similar to that in type 2 diabetes. *Cell* 96, 329–339.
- [82] Kulkarni, R.N., Holzenberger, M., Shih, D.Q., Ozcan, U., Stoffel, M., Magnuson, M.A. and Kahn, C.R. (2002) Beta-cell-specific deletion of the Igf1 receptor leads to hyperinsulinemia and glucose intolerance but does not alter beta-cell mass. *Nat. Genet.* 31, 111–115.
- [83] Xuan, S., Kitamura, T., Nakae, J., Politi, K., Kido, Y., Fisher, P.E., Morroni, M., Cinti, S., White, M.F., Herrera, P.L., Accili, D. and Efstratiadis, A. (2002) Defective insulin secretion in pancreatic beta cells lacking type 1 IGF receptor. *J. Clin. Invest.* 110, 1011–1019.
- [84] Hashimoto, N., Kido, Y., Uchida, T., Asahara, S., Shigeyama, Y., Matsuda, T., Takeda, A., Tsuchihashi, D., Nishizawa, A., Ogawa, W., Fujimoto, Y., Okamura, H., Arden, K.C., Herrera, P.L., Noda, T. and Kasuga, M. (2006) Ablation of PDK1 in pancreatic beta cells induces diabetes as a result of loss of beta cell mass. *Nat. Genet.* 38, 589–593.
- [85] Okamoto, H., Hribal, M.L., Lin, H.V., Bennett, W.R., Ward, A. and Accili, D. (2006) Role of the forkhead protein FoxO1 in beta cell compensation to insulin resistance. *J. Clin. Invest.* 116, 775–782.
- [86] Kitamura, T., Nakae, J., Kitamura, Y., Kido, Y., Biggs 3rd, W.H., Wright, C.V., White, M.F., Arden, K.C. and Accili, D. (2002) The forkhead transcription factor Foxo1 links insulin signaling to Pdx1 regulation of pancreatic beta cell growth. *J. Clin. Invest.* 110, 1839–1847.
- [87] Spiegelman, B.M. and Flier, J.S. (2001) Obesity and the regulation of energy balance. *Cell* 104, 531–543.
- [88] Despres, J.P. and Lemieux, I. (2006) Abdominal obesity and metabolic syndrome. *Nature* 444, 881–887.
- [89] Rubin, C.S., Lai, E. and Rosen, O.M. (1977) Acquisition of increased hormone sensitivity during in vitro adipocyte development. *J. Biol. Chem.* 252, 3554–3557.
- [90] Accili, D. and Taylor, S.I. (1991) Targeted inactivation of the insulin receptor gene in mouse 3T3-L1 fibroblasts via homologous recombination. *Proc. Natl. Acad. Sci. USA* 88, 4708–4712.
- [91] Cinti, S., Eberbach, S., Castellucci, M. and Accili, D. (1998) Lack of insulin receptors affects the formation of white adipose tissue in mice. A morphometric and ultrastructural analysis. *Diabetologia* 41, 171–177.
- [92] Miki, H., Yamauchi, T., Suzuki, R., Komeda, K., Tsuchida, A., Kubota, N., Terauchi, Y., Kamon, J., Kaburagi, Y., Matsui, J., Akanuma, Y., Nagai, R., Kimura, S., Tobe, K. and Kadowaki, T. (2001) Essential role of insulin receptor substrate 1 (IRS-1) and IRS-2 in adipocyte differentiation. *Mol. Cell Biol.* 21, 2521–2532.
- [93] Dowell, P., Otto, T.C., Adi, S. and Lane, M.D. (2003) Convergence of peroxisome proliferator-activated receptor gamma and Foxo1 signaling pathways. *J. Biol. Chem.* 278, 45485–45491.
- [94] Armoni, M., Harel, C., Karni, S., Chen, H., Bar-Yoseph, F., Ver, M.R., Quon, M.J. and Karnieli, E. (2006) FOXO1 represses peroxisome proliferator-activated receptor-gamma1 and -gamma2 gene promoters in primary adipocytes. A novel paradigm to increase insulin sensitivity. *J. Biol. Chem.* 281, 19881–19891.
- [95] Bluher, M., Michael, M.D., Peroni, O.D., Ueki, K., Carter, N., Kahn, B.B. and Kahn, C.R. (2002) Adipose tissue selective insulin receptor knockout protects against obesity and obesity-related glucose intolerance. *Dev. Cell* 3, 25–38.
- [96] Gelling, R.W., Morton, G.J., Morrison, C.D., Niswender, K.D., Myers Jr., M.G., Rhodes, C.J. and Schwartz, M.W. (2006) Insulin action in the brain contributes to glucose lowering during insulin treatment of diabetes. *Cell Metab.* 3, 67–73.
- [97] Obici, S., Feng, Z., Karkani, G., Baskin, D.G. and Rossetti, L. (2002) Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. *Nat. Neurosci.* 5, 566–572.
- [98] Bruning, J.C., Gautam, D., Burks, D.J., Gillette, J., Schubert, M., Orban, P.C., Klein, R., Krone, W., Muller-Wieland, D. and Kahn, C.R. (2000) Role of brain insulin receptor in control of body weight and reproduction. *Science* 289, 2122–2125.
- [99] Okamoto, H., Obici, S., Accili, D. and Rossetti, L. (2005) Restoration of liver insulin signaling in Insr knockout mice fails to normalize hepatic insulin action. *J. Clin. Invest.* 115, 1314–1322.
- [100] Burks, D.J., Font de Mora, J., Schubert, M., Withers, D.J., Myers, M.G., Towery, H.H., Altamuro, S.L., Flint, C.L. and White, M.F. (2000) IRS-2 pathways integrate female reproduction and energy homeostasis. *Nature* 407, 377–382.
- [101] Suzuki, R., Tobe, K., Aoyama, M., Inoue, A., Sakamoto, K., Yamauchi, T., Kamon, J., Kubota, N., Terauchi, Y., Yoshimatsu, H., Matsuhisa, M., Nagasaka, S., Ogata, H., Tokuyama, K., Nagai, R. and Kadowaki, T. (2004) Both insulin signaling defects in the liver and obesity contribute to insulin resistance and cause diabetes in *Irs2(-/-)* mice. *J. Biol. Chem.* 279, 25039–25049.
- [102] Lin, X., Taguchi, A., Park, S., Kushner, J.A., Li, F., Li, Y. and White, M.F. (2004) Dysregulation of insulin receptor substrate 2 in beta cells and brain causes obesity and diabetes. *J. Clin. Invest.* 114, 908–916.
- [103] Kubota, N., Terauchi, Y., Tobe, K., Yano, W., Suzuki, R., Ueki, K., Takamoto, I., Satoh, H., Maki, T., Kubota, T., Moroi, M., Okada-Iwabu, M., Ezaki, O., Nagai, R., Ueta, Y., Kadowaki, T. and Noda, T. (2004) Insulin receptor substrate 2 plays a crucial role in beta cells and the hypothalamus. *J. Clin. Invest.* 114, 917–927.
- [104] Choudhury, A.I., Heffron, H., Smith, M.A., Al-Qassab, H., Xu, A.W., Selman, C., Simmgren, M., Clements, M., Claret, M., Maccoll, G., Bedford, D.C., Hisadome, K., Diakonov, I., Moosajee, V., Bell, J.D., Speakman, J.R., Batterham, R.L., Barsh, G.S., Ashford, M.L. and Withers, D.J. (2005) The role of insulin receptor substrate 2 in hypothalamic and beta cell function. *J. Clin. Invest.* 115, 940–950.
- [105] Xu, A.W., Kaelin, C.B., Takeda, K., Akira, S., Schwartz, M.W. and Barsh, G.S. (2005) PI3K integrates the action of insulin and leptin on hypothalamic neurons. *J. Clin. Invest.* 115, 951–958.
- [106] Kitamura, T., Feng, Y., Kitamura, Y.I., Chua Jr., S.C., Xu, A.W., Barsh, G.S., Rossetti, L. and Accili, D. (2006) Forkhead protein FoxO1 mediates AgRP-dependent effects of leptin on food intake. *Nat. Med.* 12, 534–540.
- [107] Kim, M.S., Pak, Y.K., Jang, P.G., Namkoong, C., Choi, Y.S., Won, J.C., Kim, K.S., Kim, S.W., Kim, H.S., Park, J.Y., Kim, Y.B. and Lee, K.U. (2006) Role of hypothalamic Foxo1 in the regulation of food intake and energy homeostasis. *Nat. Neurosci.* 9, 901–906.
- [108] Furiyama, T., Kitayama, K., Yamashita, H. and Mori, N. (2003) Forkhead transcription factor FOXO1 (FKHR)-dependent induction of PDK4 gene expression in skeletal muscle during energy deprivation. *Biochem. J.* 375, 365–371.
- [109] Tureckova, J., Wilson, E.M., Cappelanga, J.L. and Rotwein, P. (2001) Insulin-like growth factor-mediated muscle differentiation: collaboration between phosphatidylinositol 3-kinase-Akt-signaling pathways and myogenin. *J. Biol. Chem.* 276, 39264–39270.
- [110] Liu, J.P., Baker, J., Perkins, A.S., Robertson, E.J. and Efstratiadis, A. (1993) Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). *Cell* 75, 59–72.

- [111] Coleman, M.E., DeMayo, F., Yin, K.C., Lee, H.M., Geske, R., Montgomery, C. and Schwartz, R.J. (1995) Myogenic vector expression of insulin-like growth factor I stimulates muscle cell differentiation and myofiber hypertrophy in transgenic mice. *J. Biol. Chem.* 270, 12109–12116.
- [112] Hribal, M.L., Nakae, J., Kitamura, T., Shutter, J.R. and Accili, D. (2003) Regulation of insulin-like growth factor-dependent myoblast differentiation by Foxo forkhead transcription factors. *J. Cell Biol.* 162, 535–541.
- [113] Glass, D.J. (2003) Signalling pathways that mediate skeletal muscle hypertrophy and atrophy. *Nat. Cell Biol.* 5, 87–90.
- [114] Bodine, S.C., Stitt, T.N., Gonzalez, M., Kline, W.O., Stover, G.L., Bauerlein, R., Zlotchenko, E., Scrimgeour, A., Lawrence, J.C., Glass, D.J. and Yancopoulos, G.D. (2001) Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nat. Cell Biol.* 3, 1014–1019.
- [115] Bodine, S.C., Latres, E., Baumhueter, S., Lai, V.K., Nunez, L., Clarke, B.A., Poueymirou, W.T., Panaro, F.J., Na, E., Dharmarajan, K., Pan, Z.Q., Valenzuela, D.M., DeChiara, T.M., Stitt, T.N., Yancopoulos, G.D. and Glass, D.J. (2001) Identification of ubiquitin ligases required for skeletal muscle atrophy. *Science* 294, 1704–1708.
- [116] Sandri, M., Sandri, C., Gilbert, A., Skurk, C., Calabria, E., Picard, A., Walsh, K., Schiaffino, S., Lecker, S.H. and Goldberg, A.L. (2004) Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell* 117, 399–412.
- [117] Stitt, T.N., Drujan, D., Clarke, B.A., Panaro, F., Timofeyeva, Y., Kline, W.O., Gonzalez, M., Yancopoulos, G.D. and Glass, D.J. (2004) The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors. *Mol. Cell* 14, 395–403.
- [118] Kamei, Y., Miura, S., Suzuki, M., Kai, Y., Mizukami, J., Taniguchi, T., Mochida, K., Hata, T., Matsuda, J., Aburatani, H., Nishino, I. and Ezaki, O. (2004) Skeletal muscle FOXO1 (FKHR) transgenic mice have less skeletal muscle mass, down-regulated Type I (slow twitch/red muscle) fiber genes, and impaired glycemic control. *J. Biol. Chem.* 279, 41114–41123.
- [119] Jialal, I., Crettaz, M., Hachiya, H.L., Kahn, C.R., Moses, A.C., Buzney, S.M. and King, G.L. (1985) Characterization of the receptors for insulin and the insulin-like growth factors on micro- and macrovascular tissues. *Endocrinology* 117, 1222–1229.
- [120] Vicent, D., Ilany, J., Kondo, T., Naruse, K., Fisher, S.J., Kisanuki, Y.Y., Bursell, S., Yanagisawa, M., King, G.L. and Kahn, C.R. (2003) The role of endothelial insulin signaling in the regulation of vascular tone and insulin resistance. *J. Clin. Invest.* 111, 1373–1380.
- [121] Daly, C., Wong, V., Burova, E., Wei, Y., Zabski, S., Griffiths, J., Lai, K.M., Lin, H.C., Ioffe, E., Yancopoulos, G.D. and Rudge, J.S. (2004) Angiopoietin-1 modulates endothelial cell function and gene expression via the transcription factor FKHR (FOXO1). *Genes Dev.* 18, 1060–1071.
- [122] Ross, R. (1993) The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 362, 801–809.
- [123] Owens, G.K., Kumar, M.S. and Wamhoff, B.R. (2004) Molecular regulation of vascular smooth muscle cell differentiation in development and disease. *Physiol. Rev.* 84, 767–801.
- [124] Miano, J.M. (2003) Serum response factor: toggling between disparate programs of gene expression. *J. Mol. Cell Cardiol.* 35, 577–593.
- [125] Wang, D.Z. and Olson, E.N. (2004) Control of smooth muscle development by the myocardin family of transcriptional coactivators. *Curr. Opin. Genet. Dev.* 14, 558–566.
- [126] Liu, Z.P., Wang, Z., Yanagisawa, H. and Olson, E.N. (2005) Phenotypic modulation of smooth muscle cells through interaction of Foxo4 and myocardin. *Dev. Cell* 9, 261–270.
- [127] Creemers, E.E., Sutherland, L.B., McAnally, J., Richardson, J.A. and Olson, E.N. (2006) Myocardin is a direct transcriptional target of Mef2, Tead and Foxo proteins during cardiovascular development. *Development* 133, 4245–4256.
- [128] Puig, O., Marr, M.T., Ruhf, M.L. and Tjian, R. (2003) Control of cell number by Drosophila FOXO: downstream and feedback regulation of the insulin receptor pathway. *Genes Dev.* 17, 2006–2020.
- [129] Ide, T., Shimano, H., Yahagi, N., Matsuzaka, T., Nakakuki, M., Yamamoto, T., Nakagawa, Y., Takahashi, A., Suzuki, H., Sone, H., Toyoshima, H., Fukamizu, A. and Yamada, N. (2004) SREBPs suppress IRS-2-mediated insulin signalling in the liver. *Nat. Cell Biol.* 6, 351–357.
- [130] Naimi, M., Gautier, N., Chaussade, C., Valverde, A.M., Accili, D. and Van Obberghen, E. (2007) Nuclear Foxo1 controls and integrates key signaling pathways in hepatocytes. *Endocrinology*.
- [131] Luong, N., Davies, C.R., Wessells, R.J., Graham, S.M., King, M.T., Veech, R., Bodmer, R. and Oldham, S.M. (2006) Activated FOXO-mediated insulin resistance is blocked by reduction of TOR activity. *Cell Metab.* 4, 133–142.
- [132] Teleman, A.A., Chen, Y.W. and Cohen, S.M. (2005) Drosophila melted modulates FOXO and TOR activity. *Dev. Cell* 9, 271–281.
- [133] Cao, Y., Kamioka, Y., Yokoi, N., Kobayashi, T., Hino, O., Onodera, M., Mochizuki, N. and Nakae, J. (2006) Interaction of FoxO1 and TSC2 induces insulin resistance through activation of the mammalian target of rapamycin/p70 S6K pathway. *J. Biol. Chem.* 281, 40242–40251.