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The role of FOXO transcription factors in the development of type 2 diabetes and related potential therapeutic possibilities

ABSTRACT

Forkhead box class O (FOXO) family member proteins are key transcription factors for maintaining the intracellular homeostasis in response to changes in the internal and external environment. They participate in the control of such cellular processes as proliferation, cell cycle, apoptosis, glucose and lipid metabolism, and oxidative stress response. Altered expression and activity of these factors are associated with development of metabolic disturbances, primarily type 2 diabetes. Understanding of the role of FOXO in the pathophysiology of these abnormalities will enable appropriate steps to prevent their development and to create therapies targeted at the disturbances underlying type 2 diabetes and metabolic syndrome. In the present article, we summarized the current knowledge about the physiology and pathophysiology of these transcription factors and described their role in the development of diabetes and functioning of various organs. We focused on their role in progression of diabetes and indicated potential targets for future therapeutic interventions. (Clin Diabetol 2021; 10; 3: 290–298)

Key words: diabetes, FOXO transcription factors, β-cell failure, glucose metabolism, lipid metabolism, insulin signalling pathway, oxidative stress

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Introduction

Forkhead box class O (FOXO) proteins are nuclear transcription factors which participate in the regulation of various cellular processes such as proliferation, cell cycle and apoptosis, DNA repair, cellular differentiation, metabolism (particularly of glucose and lipids), and resistance to oxidative stress [1–3]. This protein family includes 4 members: FOXO1, FOXO3A, FOXO4 i FOXO6. They mediate reaction cascades triggered by insulin and insulin-like growth factor-1 (IGF-1) intended to inhibit selected cell functions [1], which serves to maintain the intracellular homeostasis in response to changes in the internal and external environment [4]. The structure of FOXO proteins is shown in Figure 1. FOXO1 and FOXO3A are expressed in nearly all body tissues, while FOXO4 is expressed mostly in the muscles, kidneys, and the large intestine, and FOXO6 is present mainly in the brain and muscles [5]. The activity of these proteins is largely regulated by posttranslational modifications (mainly phosphorylation, acetylation, and glycosylation), the effects of which involve three mechanisms: FOXO transport from or to the nucleus, changes in the affinity of FOXO to DNA, and modification of the transcription activity of FOXO [3, 6]. The regulation of FOXO activity by phosphorylation, which serves as the main regulatory mechanism, is shown in detail in Figure 2. Mutations in the genes coding for FOXO proteins or changes in the expression of these proteins lead to the development of diabetes and malignancies or shortening of the lifespan in mammals [7].

In the present article, we described the general mechanism of action of FOXO proteins in various organs, with particular attention to their effect on glucose and lipid metabolism, as also shown in Figure 3. This is followed by a discussion on the role of FOXO proteins

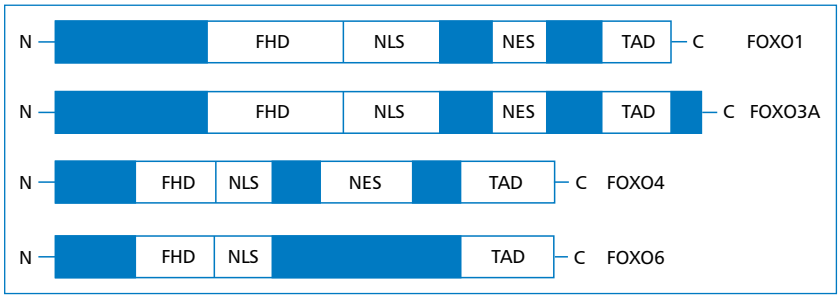

Figure 1. Structure of FOXO proteins. FOXO proteins show marked homology and include four functional domains: forkhead domain (FHD), nuclear localization sequence (NLS), nuclear export sequence (NES), and transactivation domain (TAD). The FHD domain is responsible for transcription factor binding to DNA of the chromatin. The NLS fragment is necessary to retain FOXO proteins in the nucleus where they affect expression of the target genes. The NES sequence allows transporter protein binding to FOXO and their translocation to the cytoplasm, which terminates the effect of transcription factors on the target genes. Finally, the TAD domain participates in increasing promoter activity. The effectiveness of NLS and NES domains is modulated by various proteins, which contributes to the circulation of FOXO factors between the nucleus and the cytoplasm [65, 66]. Blue fragments indicate aminoacid sequences which are not parts of the above domains but play a major role in the structure of FOXO proteins as potential targets for the proteins which modify the activity of these transcription factors. The length proportions between various fragments of the FOXO proteins do not reflect their relative true sizes

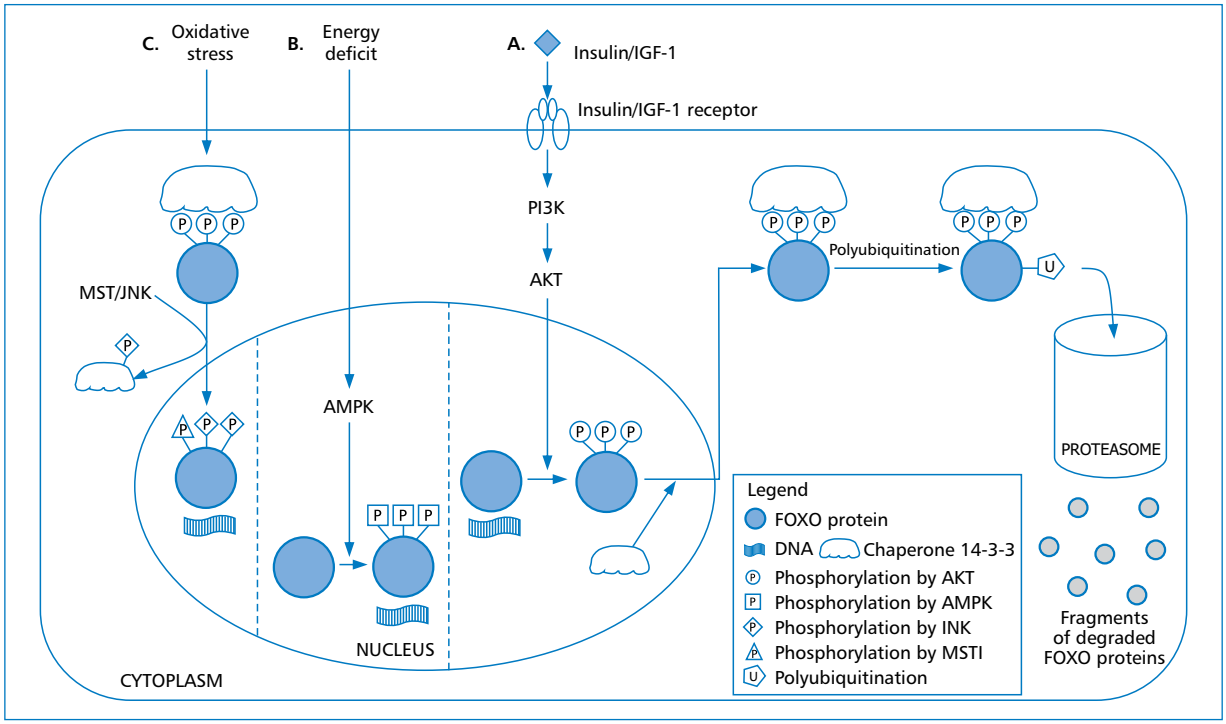

Figure 2. Regulation of FOXO activity by phosphorylation. Phosphorylation of FOXO takes place at various sites and is catalysed by various kinases, which affects the location of these proteins in the nucleus, their affinity to DNA, and transcription activity [2, 3]. **A.** The major factors participating in this posttranslational modification mechanism are insulin and insulin-like growth factor 1 (IGF-1) which initiate the signalling pathway of phosphoinositide 3-kinase (PI3K) and protein kinase B (Akt). This leads to phosphorylation of FOXO, facilitating binding of chaperone 14-3-3 protein to FOXO, which in turn permits active transport of FOXO to the cytoplasm, blocks their return to the nucleus, and leads to ubiquitin-dependent degradation in proteasomes [67]. As a result, transcription activity of FOXO is inhibited. In addition, Akt-mediated phosphorylation of FOXO impairs its interaction with DNA [68]. **B.** In the settings of insulin and IGF-1 absence and in response to energy deficit, adenosine monophosphate-activated protein kinase (AMKP) phosphorylates nuclear FOXO at other sites than Akt and activates transcription but has no effect on intracellular translocation of FOXO [69]. **C.** In the settings of oxidative stress, c-Jun N-terminal kinase (JNK) phosphorylates FOXO and induces its import from the cytoplasm to the nucleus [70]. In addition, JNK may phosphorylate chaperone 14-3-3 protein, which leads to its dissociation from FOXO in the cytoplasm and FOXO transport to the nucleus [71]. Another kinase, serine/threonine-protein kinase 4 (mammalian STE20-like kinase-1, MST1), phosphorylates FOXO, blocking its interaction with chaperone 14-3-3 protein and inducing FOXO translocation to the nucleus [72]. In addition, MST1 activates cellular JNK pathway [73]

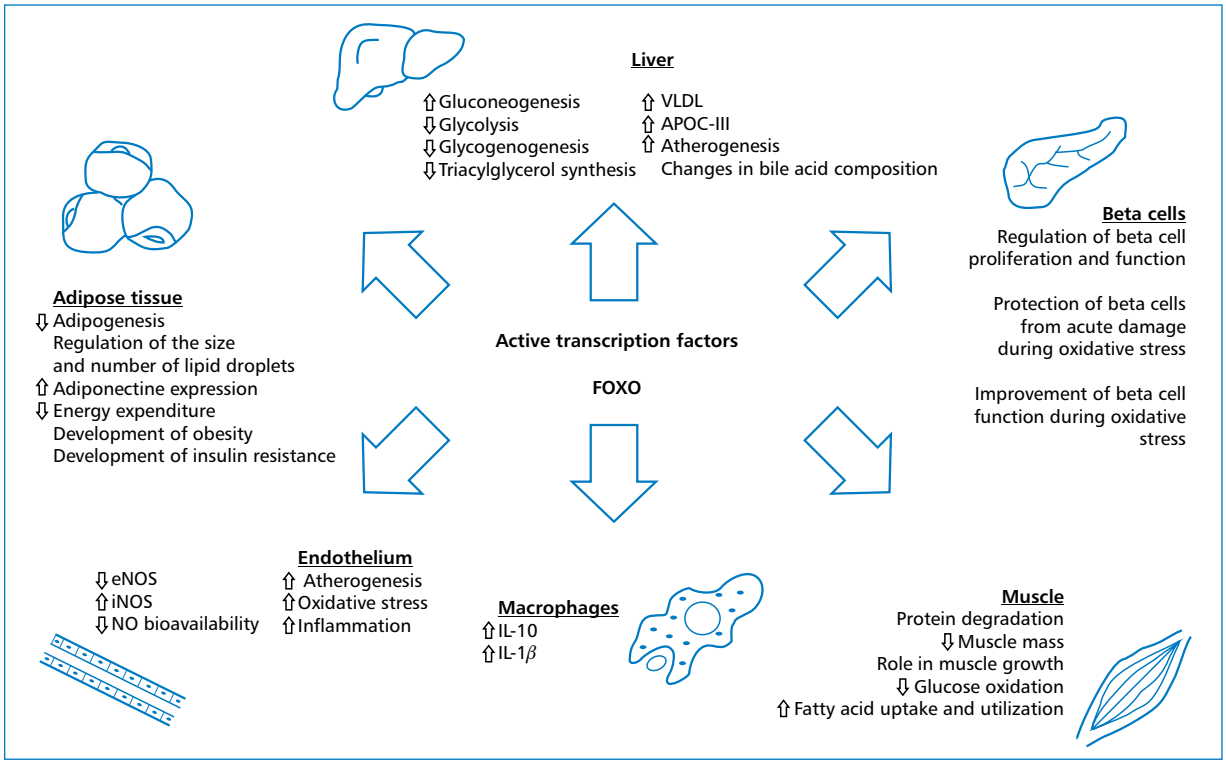

Figure 3. Effects of active FOXO transcription factors. APO, apolipoprotein; eNOS, endothelial nitric oxide synthase; IL, interleukin; iNOS, inducible nitric oxide synthase; NO, nitric oxide; VLDL, very low density lipoproteins

in the pathogenesis of type 2 diabetes and its complications to indicate potential targets for future therapies in the management of diabetes.

Liver

FOXO1 plays a key role in the regulation of hepatic synthesis of glucose and lipids [8]. During fasting, FOXO1 is activated and interacts with peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α), the major regulator of enzymes responsible for gluconeogenesis, including glucose 6-phosphatase (G6P) and phosphoenolpyruvate carboxykinase (PEPCK) [9]. This leads to transcription of genes coding for these enzymes and increased hepatic glucose production [10]. In addition, by blocking activation of the hepatocyte nuclear factor 4 (HNF-4), FOXO1 prevents activation of the gene coding for glucokinase, the key enzyme of glycolysis [11, 12]. In contrast, in the settings of satiety, insulin leads to inactivation of FOXO1, which results in suppression of gluconeogenesis, and activation of glucokinase, which increases glycogenogenesis and triglyceride synthesis. This explains why an increased hepatic triglyceride synthesis is observed in the settings of insulin resistance and very early stages of type 2 diabetes, characterized by hyperinsulinaemia to maintain normoglycaemia [13]. This observation suggests that FOXO transcription factors evolved as an evolutionary mechanism to control the metabolic switch between glucose and lipid oxidation depending on food availability.

Studies showed that constitutive hepatic FOXO1 expression leads to an increase in fasting blood glucose level and development of hepatic insulin resistance [8], while loss of hepatic FOXO1 function contributes to a decrease in gluconeogenesis during fasting by about 50% [14] and occurrence of fasting hypoglycaemia [11, 15]. Inhibition of FOXO1 markedly reduced glycogenolysis and gluconeogenesis in mice with hyperinsulinaemia and normoglycaemia, leading to an increased tissue insulin sensitivity. Rodents with diabetes induced by inhibiting any step of the insulin signalling did not develop the diabetic phenotype if the FOXO1 gene was concurrently inhibited [15,16]. This may be explained by a reduced hepatic expression of gluconeogenesis genes and an increased expression of insulin sensitivity genes in the adipocytes [17].

Activation of FOXO1 occurs in the settings of cellular stress, including stress induced by free oxygen radicals [18]. During fasting, adenosine monophosphate-activated protein kinase (AMPK) is activated via a glucagon-stimulated pathway, which ultimately leads to increased FOXO transcriptional activity and induction of transcription of gluconeogenesis genes [19].

FOXO proteins also play an important role in lipid metabolism. FOXO1 participates in insulin-dependent hepatic regulation of production of very low density lipoproteins (VLDL) and the half-time of VLDL survival in the circulation. These effects are mediated by stimulating synthesis of apolipoprotein C-III (APO C-III) and microsomal triglyceride transfer protein (MTTP) [14] which are major factors in triglyceride circulation during fasting. In the settings of insulin deficiency, active FOXO1 increases transcription of the MTTP gene, coding for an enzyme that is critical for hepatic VLDL production, leading to increased VLDL secretion, and also increases the transcription activity and hepatic secretion of APO C-III [20]. The latter apolipoprotein regulates various steps of the metabolism of triglyceride-rich lipoproteins (TRL), e.g., inhibits hepatic TRL uptake and decreases TRL conversion to low density lipoproteins (LDL) [21], and inhibits lipoprotein lipase [20] and hepatic lipase [22]. Ultimately, the half-time of VLDL survival in the circulation is increased. During the resorption period, insulin inactivates FOXO1 and thus abolishes its effect on MTTP and APO C-III. In the settings of insulin resistance, FOXO1 remains active and continues to affect MTTP and APO C-III, which is one of the drivers for hypertriglyceridaemia. Other studies suggest that no FOXO isoform is required for hepatic expression of APO C-III, and normalization of serum APO C-III level during intensive insulin therapy in diabetic mice is likely related to increased hepatic remnant uptake [23]. Elevated APO C-III level in diabetes probably contributes to the accumulation of atherogenic lipoproteins containing APO C-III, apolipoprotein E (APO E) and apolipoprotein B (APO B) in the vessel wall, which facilitates monocyte recruitment and accumulation of macrophage-derived foam cells. Studies showed that reducing APO C-III level using antisense oligonucleotides (ASO) led to a significant reduction of atherogenesis, likely due to a reduction of proatherogenic lipoprotein levels [23]. This may constitute a therapeutic target in diabetic patients.

The FOXO1 transcription factor also plays a key role in the synthesis of bile fatty acids by regulating CYP8B1 (12α-hydroxylase) and potentially also other genes participating in their synthesis. Bile acids reduce triglyceride levels [24] but their 12-hydroxylated derivatives are less effective in inhibiting triglyceride and cholesterol synthesis compared to non-12-hydroxylated bile acids [25]. In diabetes, due to lacking insulin effect on the tissues, FOXO1 remains active, leading to higher levels of 12α-hydroxylated fatty acids. This effect may by reversed by a pharmacological intervention, constituting another possibility of an intervention targeted at metabolic disturbances in patients with type 2 diabetes [24].

Adipose tissue

Adipose tissue participates in the regulation of energy homeostasis of the body. In adipocytes, active FOXO1 inhibits adipogenesis by preventing differentiation of the preadipocyte cellular line [26] at early and late stages by induction of the cell cycle inhibitor p21 [27]. In addition, FOXO1 regulates the expression and activity of peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer-binding protein α (C/EBPα), two key transcription factors participating in adipogenesis. The expression and activity of PPARγ are directly inhibited by FOXO1 [28]. FOXO1 regulates the size and number of lipid droplets in the adipose tissue by regulating the autophagy process mediated by fat-specific protein 27 (FSP27), and its silencing might potentially inhibit adipocyte differentiation and growth of lipid droplets [29], which would translate to the prevention of hypertrophy and growth of adipose tissue [30] and might open up new possibilities in the management of obesity. FOXO1 also interacts physically with C/EBPα and promotes expression of adiponectin [26]. The latter has a beneficial effect on the regulation of glucose and lipid metabolism in the muscles, liver, adipose tissue and other tissues. In the adipose tissue, adiponectin regulates preadipocyte proliferation, adipocyte differentiation, insulin sensitivity, and lipid storage [31]. Reduced adiponectin expression compared to healthy controls was shown both in mice with obesity induced by high-fat diet and mice with diabetes, which was explained by abnormal FOXO1-C/EBPα-mediated regulation [32].

FOXO1 also participates in the regulation of thermogenesis in the adipose tissue, mediated by zinc finger protein 238 (ZFP238). In the absence of ZFP238, FOXO1 may block expression of the uncoupling protein 1 (UCP1) gene. This contributes to a reduction in energy expenditure and development of obesity and insulin resistance. Upon exposure to cold, ZFP238 binds with FOXO1 and blocks its transcription activity in the adipocytes, which ultimately leads to the activation of UCP1 gene and transformation of white to beige adipose tissue [33]. Studies showed that absent FOXO1 gene expression in the white adipose tissue in mice helps maintain normal glucose tolerance and insulin sensitivity and increased energy expenditure on both normal and high-fat diet, and absence of FOXO1 in brown adipose tissue increases oxygen uptake and expression of PGC1α and UCP1 genes which favour aerobic metabolism [34]. These data suggest that FOXO1 regulates energy homeostasis in the white adipose tissue, and energy expenditure in the brown adipose tissue.

Pancreatic beta cells

FOXO1 regulates beta cell function in two ways. On one hand, FOXO1 inhibits beta cell proliferation in insulin resistance conditions and during their differentiation in the developing fetal pancreas. On the other hand, it protects beta cells from damage resulting from oxidative stress associated with glucose and lipid overload [35]. FOXO1 ablation at various time points results in the development of markedly different phenotypes which suggests that this protein exerts different physiological functions at various stages of pancreas development [36]. In the animal models of FOXO1 function loss, beta cell proliferation and function are inhibited [17].

In healthy individuals, FOXO transcription factors remain inactive in beta cells and become activated in response to hyperglycaemia [37]. In advanced type 2 diabetes, loss of FOXO2 in beta cells ensues concurrently with the loss of insulin secretion capability [38]. At an early stage of beta cell damage, FOXO transcription factors become inactivated. Animal studies showed that this is due to an attempt to maintain a balance between acetylcoenzyme A (Acetyl-CoA) synthesis from glucose versus from lipids in the early phase of diabetes. Acetyl-CoA is required for mitochondrial oxidation. In the early phase of diabetes, glucose oxidation is impaired compared to normally functioning beta cells, while lipid oxidation is significantly increased [39]. Excess lipid oxidation leads to formation of toxic products, mostly superoxides, and impaired synthesis of adenosine triphosphate, calcium mobilisation, and insulin secretion [40].

Chronic oxidative stress in diabetes is probably induced by toxicity of glucose, the intracellular level of which exceeds the glycolytic capacity of beta cells. In these conditions, glucose undergoes enolization which results in synthesis of superoxide anions and induction of apoptosis of these cells [41]. Oxidative stress promotes FOXO1 activation which may protect beta cells against acute metabolic disturbances and damage induced by short-term oxidative stress but does not protect from chronic alterations related to long-lasting hyperglycaemia [42]. FOXO1 also stimulates expression of catalase and glutathione peroxidase in beta cells, which leads to an increased survival and better functioning of these cells during exposition to oxidative stress [43].

Recent studies indicate a high potential for clinical use of FOXO proteins in regenerative medicine. Manipulations with FOXO transcription factors might transform non-beta cells into insulin-producing cells. Inactivation of FOXO1 in intestinal endocrine cells results in expansion of enteroendocrine progenitor cells containing neurogenin-3 and appearance of functional insulin-producing cells which show expression of all mature pancreatic beta cells markers including C-peptide and release insulin in response to physiological and pharmacological triggers. These cells are able to mitigate the course of streptozotocin-induced diabetes [44]. Thus, FOXO1 inhibition targeted to the intestine might be a promising strategy for the management of diabetes in humans. FOXO proteins are necessary to maintain the process of beta cell differentiation, and development of diabetes is associated with a significant loss of FOXO function which leads to de-differentiation of beta cells. FOXO stabilizes insulin-producing pancreatic islet cells. De-differentiation may be one of the major reasons for damage of these cells and their transformation into non-beta endocrine cells, which suggests that management of diabetes should focus on maintaining adequate differentiation of beta cells [38].

Muscles

FOXO1 promotes myoblast proliferation, fusion of mononuclear cells into myotubules and degradation of muscle fibres [45]. In mice with overexpression of FOXO1, genes characteristic for slowly-contracting muscles become downregulated which suggests that this transcription factor promotes differentiation of muscle cell lines [46]. Muscle-specific FOXO1 ablation results in a change of the muscle fibre type towards rapidly-contracting muscle fibres containing myoblast determination protein (MYO-D), with concurrent reduction in the number of slowly-contracting fibres containing myogenin [47]. In addition, FOXO1 inhibits MYO-D--dependent myogenesis in C212 myoblast colonies [46]. PGC-1α, a transcription cofactor that plays a major role, among others, in the biogenesis of mitochondria and fatty acid oxidation in the skeletal muscle, is also of a key importance in the conversion of muscle fibres from rapidly-contracting to slowly-contracting ones during adaptation to aerobic metabolism [48]. PGC-1α stimulates expression of FOXO6 in murine skeletal muscle in response to a low-intensity aerobic exercise [49].

In vivo studies on inactivation or overexpression of FOXO1 indicate that these manipulations have a significant effect on the skeletal muscle mass. In mice with overexpressed FOXO1, dysglycaemia developed due to a reduction in muscle mass [46]. This effect was related not only to inhibition of myogenesis but also to generalized muscle atrophy resulting from muscle fibre degradation [50]. Muscle-specific FOXO deletion protects diabetes-induced muscle atrophy by blocking protein degradation mediated by proteasomes and lysosomes, while it has no effect on glucose homeostasis. FOXO-dependent degradation of muscle proteins was

also shown to be initiated after 8 hours of insulin deficiency in patients with type 1 diabetes. FOXO proteins are thus major regulators of diabetes-induced muscle atrophy and constitute a potential therapeutic target to prevent muscle atrophy in diabetic patients [51].

Muscle-specific FOXO deletion in mice leads to an increase in muscle size by about 40% in both females and males. Male mice with FOXO1, FOXO3A, and FOXO4 ablation showed an impaired insulin effect on the muscular tissue, while such an effect was not confirmed in female mice with the same ablation. Insulin resistance in the muscles of these male mice was functionally significant, as insulin-stimulated glucose uptake in the muscles was significantly lower compared to the controls. These data suggest that FOXO transcription factors favour preserving insulin sensitivity in male mice. In both male and female mice, deletion of FOXO1, FOXO3A, and FOXO4 in the muscles also leads to muscle hypertrophy which limits the growth of adipose tissue and dysglycaemia in the setting of concomitant diet-induced obesity. These studies indicate that inhibiting FOXO in the muscles may be an important therapeutic target to mitigate insulin resistance in the muscles and reduce metabolic complications of obesity [51].

FOXO transcription factors also affect glucose and lipid metabolism in the skeletal muscle by exerting an effect on the expression of three enzymes responsible for switch from carbohydrate oxidation, the main source of energy in the postresorption period, to fatty acid oxidation [52]. FOXO stimulate uptake and utilization of fatty acids in the muscles by regulating fatty acid transporter protein CD36 and lipoprotein lipase [42]. In the settings of energy deficiency, FOXO1 increases expression of pyruvate dehydrogenase kinase 4 (PDK4), an enzyme that reduces the activity of pyruvate dehydrogenase, thus reducing glucose oxidation [52]. At the same time, overexpression of FOXO1 increases expression of lipoprotein lipase in C2C12 myoblasts and serum level of fatty acid translocase, which facilitates free fatty acid uptake by the skeletal muscle [53]. In the postresorption period, FOXO is upregulated to maintain energy homeostasis by preferential utilization of lipids over carbohydrates as the source of energy. In contrast, the role of FOXO during fasting is to provide energy by inducing muscle protein catabolism, which leads to muscle loss and atrophy and underlies glucose intolerance in the conditions of insulin resistance [54].

Endothelial cells

Endothelial dysfunction leads to atherogenesis by reduced nitric oxide availability, the presence of inflammation, and generation of superoxide [55]. Genetically impaired insulin signalling in the endothelial cells is associated with reduced inactivation of FOXO1. This results in abnormal nitric oxide release and suggests that FOXO1 may be responsible for the genetic predisposition to the development of endothelial dysfunction and cardiovascular disease [56]. FOXO1 inhibits activity of endothelial nitric oxide synthase (eNOS) [57] and increases activity of inducible nitric oxide synthase (iNOS) [58], which suggests a proatherogenic role of these transcription factors in the conditions of insulin resistance in type 2 diabetes [54]. Studies showed that increased FOXO1 activity in the endothelial cells was associated with reduced insulin sensitivity in the adipose tissue in obese individuals and led to impaired eNOS activation [59]. In addition, overexpression of nuclear FOXO1 in the endothelium was associated with reduced nitric oxide availability, increased oxidative stress and inflammation, and reduction of antioxidative defences, all promoting apoptosis [60]. It was shown that pharmacological FOXO1 blockade may improve insulin-dependent vasodilatation in the muscle microcirculation and increase muscular blood flow [59].

Endothelial FOXO proteins also favour insulin resistance on a high-fat diet, which may in part result from the fact that these proteins create an antiangiogenic and proinflammatory milieu in the skeletal muscle. These discoveries also provided information on the role of microvascular dysfunction in the progression of type 2 diabetes. During prolonged high-fat diet, the endothelial FOXO1 pool increases, which inhibits angiogenesis and leads to progression of the prediabetic phenotype. Blockade of endothelial FOXO proteins significantly modifies the skeletal muscle microcirculation and preserves normal response to insulin [61].

In other studies, mice with endothelial cell-specific ablation of FOXO1, FOXO3A and FOXO4 showed an increased eNOS-dependent nitric oxide synthesis in the hepatic sinuses and demonstrated impaired glucose tolerance and hepatic insulin resistance when fed a standard diet. These findings suggest that excess nitric oxide produced by eNOS reduces hepatic insulin resistance and plays a role in the pathophysiology of the development of obesity-related insulin resistance at its early stage. Complementary studies on hyperinsulinaemia yielded similar results, and pharmacological eNOS blockade in a murine model of early insulin resistance partially restored glucose tolerance [62].

Macrophages

In macrophages, FOXO1 transcription factor is a key regulator of inflammation, mediating regulation of interleukin-10 (IL-10) gene expression during classical lipopolysaccharide (LPS)-dependent activation. Studies indicate an association between FOXO1-dependent

macrophage phenotype and hyperglycaemia and hyperlipaemia, major components of the metabolic syndrome. FOXO1 gene expression is reduced with exposure to high glucose levels. Macrophages develop a proinflammatory phenotype with a reduced IL-10 level, which is associated with the pathophysiology of obesity, diabetes, and atherosclerosis. This confirms previous suggestions that FOXO1 participates in the regulation of macrophage phenotype by a permissive effect for the IL-10 gene. These findings indicate that FOXO1 is factor underlying progression of inflammation in response to metabolic disturbances of cellular stress [63].

Macrophages derived from mice with bone marrow-specific ablation of FOXO1, FOXO3A and FOXO4 showed a reduced sensitivity to cholesterol-induced apoptosis and elevated levels of oxidative stress markers. In addition, these cells were characterized by an impaired insulin signalling, which is the hallmark of insulin resistance [54]. Other studies showed that FOXO1 protein stimulated expression of proinflammatory interleukin 1β (IL-1β) and enhanced the inflammatory response in mature macrophages, which suggests that FOXO1 enhances innate immunity mechanisms in macrophages [64].

Overall, these findings indicate a significant role of bone marrow-derived FOXO factors in the context of atherogenesis and development of metabolic disease.

Conclusions

Ubiquitous presence and multidirectional effects of FOXO transcriptional factors indicate their key role in the regulation of cellular metabolism, in particular glucose and lipid metabolism, and maintenance of intracellular homeostasis. Interventions to modulate FOXO expression and activity seem a promising approach to prevent and modify the course of civilization-associated diseases such as diabetes, metabolic syndrome, obesity, and atherosclerosis. Further studies are required to elucidate the physiological importance of these proteins and their role in the pathophysiology of selected disease conditions, with the general aim of developing new therapies targeted at their underlying causes and not only clinical manifestations.

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Conflict of interests

The authors do not declare any conflict of interests.

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