Role of TXNIP Biology in Glucose Metabolism

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Abstract

Skeletal muscle insulin resistance is a major contributor to the natural history of type 2 diabetes mellitus. A common underpinning that exists behind the mechanisms of insulin resistance is the overproduction of reactive oxygen species, which may be exacerbated by a protein target known as thioredoxin-interacting protein (TXNIP). TXNIP is a member of the α-arrestin protein family that is capable of suppressing glucose metabolism in several tissues. Cross-sectional analysis reveals that individuals with insulin resistance, impaired glucose tolerance, and type II diabetes have elevated protein and mRNA expression of TXNIP in skeletal muscle compared to lean healthy controls, which negatively correlates with insulin sensitivity. The goal of this review is to explore TXNIP’s role in glucose metabolism and provide mechanisms through which it can be regulated to provide potential targets for future research and therapeutic interventions for the treatment of insulin resistance.

Keywords

Diabetes, Insulin resistance, TXNIP, Exercise

Introduction

Type II diabetes (T2DM) is a debilitating disease that places significant economic burden on the healthcare system with over $245 billion in annual costs affecting over 30 million Americans [1]. T2DM is characterized by chronic hyperglycemia which promotes oxidative stress and inflammation in multiple tissues. If left untreated, T2DM can lead to an increased risk of heart disease, kidney failure, and augmented cognitive decline [2-5]. Although T2DM requires the coordinated dysfunction of several tissues, understanding the molecular mechanisms that promote insulin resistance in skeletal muscle should be a chief concern, as this tissue is the primary site for insulin-mediated glucose metabolism [6]. A protein that has gained recent attention in relation to the regulation of insulin action at the level of the skeletal muscle, is thioredoxin-interacting protein (TXNIP).

TXNIP description and domain analysis

TXNIP, also referred to as Vitamin-D upregulated protein 1 (VDUP-1), was first discovered in HL-60 leukemia cells and shown to be upregulated in response to treatment with 1,25-dihydroxyvitamin D$_3$ [7]. Subsequent investigations utilizing two-hybrid yeast analysis revealed that TXNIP is capable of binding to cysteine residues located within the active site of antioxidant thioredoxin-1 (TRX-1) and 2 (TRX-2) and inhibiting

List of Abbreviations

T2DM: Type II Diabetes; TXNIP: Thioredoxin Interacting Protein; REDD1: Regulated in Development and DNA Damage Responses 1; TRX: Thioredoxin; NGT: Normal Glucose Tolerant; IGT: Impaired Glucose Tolerant; ASK1: Apoptotic Signaling Kinase; PTEN: Phosphatase Tensin Homolog 10; REF-1: Redox Factor-1; HIF-1: Hypoxia-Inducible Factor-1a; NF-kB: Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells; AP-1: Activator Protein-1; NRF-2: Nuclear Factor E2-Related Factor 2; GR: Glucocorticoid Receptor; ER: Estrogen Receptor; ETC: Electron Transport Chain; AICAR: 5-Aminoimidazole-4-Carboxamide Ribonucleotide; PBMC: Peripheral Blood Mononuclear Cells; PP2A: Protein Phosphatase 2; AKT: Protein Kinase B; P38 MAPK: P38 Mitogen-Activated Protein Kinase; FOXO1: Foxhead Box 01 Transcriptional Factor; CCP: Clathrin Coated Pits; CHREBP: Carbohydrate Response Element Binding Protein; P300: Histone Acetyltransferase p300; PA: Palmitic Acid; NAC: N-Acetyl-L-cysteine; AMPK: AMP-Activated Protein Kinase; SIRT1: Sirtuin 1; NLRP3: NOD-Like Receptor P3; PERK: PKR-like ER Resident Kinase; ATF6: Activating Transcription Factor 6

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their ability to scavenge reactive oxygen species thus promoting oxidative stress [8]. Although TXNIP belongs to the α-arrestin protein family, the active cysteine residues that enable its interaction with TRX are not present in other members of the family, indicating that TXNIP has the distinct ability to bind to the active site of TRX and affect the redox environment [9]. Spindel, et al. [10] conducted three-dimensional (3D) analysis of TXNIP and determined that TXNIP contains several domains that are conserved amongst other members of the α-arrestin family and are necessary for its redox-independent functions. Within the n-terminus there are two SH3-binding domains which are known to interact with non-tyrosine kinase Src, and a mitogen activated protein kinase kinase kinase 5 (MAP3K-5) [10-12]. Conversely, within the C-terminus lies three SH3-binding domains and two PPxY motifs. The PPxY motifs are necessary for TXNIP to interact with E3 Ubiquitin Ligase, Itch, enabling it to undergo polyubiquitination and subsequent proteasome degradation [13]. Recent evidence suggests that following phosphorylation of a specific serine residue, ser308, TXNIP undergoes a conformational change that increases its susceptibility to ubiquitination via Itch, augmenting its rate of proteasome degradation [14]. TXNIP also contains two additional domains including immune-receptor tyrosine-based inhibition domain (ITIM) and chromatin maintenance region 1 (CRM1) [10]. ITIM mediates TXNIP’s interaction with tyrosine phosphatases in order to regulate the intracellular activity of membrane-bound receptors [15]. CRM1 mediates the interaction with hypoxia-inducible factor 1-α (HIF1-α) and ubiquitin ligase von Hippel-Lindau protein (pVHL), leading to the exclusion of HIF1-α from the nucleus and degradation in the cytosol [16].

Role of TXNIP in glucose metabolism

TXNIP’s role in regulating metabolism was first investigated in HcB-19 mice used as a preclinical model to study familial hypercholesterolemia in humans. Interestingly, the hypcholesteremia was a result of a spontaneous mutation on the TXNIP gene leading to systemic reductions at the mRNA levels. As a result, HcB-19 mice had lower basal CO₂ production, increased triglyceride synthesis, and decreased flux of free fatty acids (FFA) through the tricarboxylic acid (TCA) cycle [17]. Considering that mitochondrial FFA flux may be a contributor to insulin resistance, Chutkow, et al. [18] performed additional experiments to determine the effect of a systemic TXNIP knockout on insulin sensitivity and substrate utilization. TXNIP null mice had significant increases in adiposity compared to their wild-type littermates, however, they were protected from high-fat diet induced insulin resistance. Concomitantly, TXNIP-null mice saw improvements in clamp-derived glucose disposal, glycolytic flux and glycogen synthesis, indicating that TXNIP is a negative regulator of whole body glucose metabolism [18].

To delineate TXNIP’s role further at the tissue and cellular level, a preclinical model with liver-specific deletion of TXNIP was developed. With TXNIP deletion, hepatic glucose production was blunted which led to hypoglycemia during the fasted state. However, in response to a glucose challenge, liver-specific TXNIP knockout mice were incapable of achieving greater glucose clearance rates compared to their wildtype littermates. This finding is in contrast with systemic knockout models indicating that although fasting glucose homeostasis is modulated by hepatocyte TXNIP levels; postprandial glucose tolerance may require modulation of TXNIP biology in other tissues and cell types [19].

DeBalsi, et al. [20] developed a skeletal muscle-specific TXNIP knockout (SKM -/-) murine model. Comparisons between the whole-body knockout, skeletal muscle-specific knockout, and wild type revealed that glucose tolerance was enhanced (i.e. lowered circulating glucose concentrations) in the whole-body knockout model following an intraperitoneal glucose tolerance test. Interestingly, these improvements were preserved in the SKM (-/-) specific knockout indicating that skeletal muscle expression of TXNIP contributes significantly to whole-body glucose homeostasis. Although SKM (-/-) specific TXNIP deficiency improved glucose tolerance, the mice also experienced reduced mitochondrial respiration, substrate oxidation, mitochondrial protein markers, and aerobic exercise capacity. These data suggest that TXNIP serves as a metabolic switch between oxidative and glycolytic metabolism, and must be regulated appropriately to achieve proper balance between energy systems.

Despite extensive work conducted on TXNIP expression and glucose metabolism, the mechanism through which TXNIP exerted these effects was still largely unknown. Olsowski, et al. [21] provided some initial insight by demonstrating in pancreatic β-cells that TXNIP can interact and mediate the activation of the nod like receptor P3 (NLRP3) inflammasome, which is a large multimeric protein complex, capable of inducing endoplasmic reticulum (ER) stress, IL-1β expression, and apoptotic cell death. These findings were substantiated in microvascular endothelial cells by demonstrating that silencing of TXNIP ameliorated NLRP3 activation in response to oxidative stress [22]. Further, NLRP3 inflammasome activation may be required for lipid-induced insulin resistance in vivo [23], but TXNIP’s role has yet to be elucidated in this context. Investigation may be warranted however, considering lipid-induced insulin resistance in C2C12 skeletal muscle cells requires TXNIP, as RNA interference prevented insulin resistance following treatment with palmitic acid [24]. In addition to the inflammasome, TXNIP can act as an adaptor protein to link GLUT1 with clathrin coated pits (CCPs) to increase the rate of GLUT1 endocytosis, limiting glucose uptake in hepatocytes [14]. Furthermore, Waldhart, et al. expanded the endocytosis mechanism to include GLUT4 transporters, which was conducted in 3T3L1 adipocytes,
however, this mechanism has yet to be investigated in skeletal muscle biology. Nonetheless, these mechanisms provide a direct link between TXNIP expression and blunted basal and insulin-mediated glucose uptake capacity [25].

**Regulation of TXNIP: Transcriptional and posttranslational mechanisms**

As mentioned previously, the first known regulation of TXNIP gene expression was discovered in 1,25-dihydroxyvitamin D3-treated HL-60 leukemia cells [7]. Subsequent investigations revealed that TXNIP is upregulated in response to various stimuli including oxidative stress, ER stress, hypoxia, lactic acidosis, as well as intracellular calcium and glucose accumulation [21,26-29]. Specifically, the mechanism through which ER stress mediate TXNIP expression occurs via activation of enzymes that mediate the unfolding protein response including inositol requiring enzyme 1 (IRE1), PKR-like ER resident kinase (PERK), activating transcription factor 6 (ATF6), and carbohydrate-response element binding protein (ChREBP) [21]. Activation of ChREBP provides a point of convergence between these various stimuli. For example, calcium flux can increase the nuclear translocation of ChREBP. However, calcium channel blockers have been shown to be efficacious in preventing overexpression of TXNIP via inactivation of the phosphatase, calcineurin, which is responsible for dephosphorylating ChREBP to enable its nuclear translocation [30]. Additionally, β-cells treated with high glucose increased TXNIP expression, however the investigators determined by gene silencing, that the effect was dependent on the recruitment of transcription factor, ChREBP and hormone acetyltransferase p300 (p300), to its promoter site [28]. Kibbe, et al. [31] provided further insight into this mechanism, determining that FOXO1 is a transcriptional repressor of TXNIP that can be outcompeted by ChREBP in order activate gene expression in response to glucose treatments. This mechanism may be cell-type specific however, as glucose-induced TXNIP expression in endothelial cells is mediated via activation of p38 mitogen-activated protein kinase (p38 MAPK) and forkhead box 01 transcriptional factor (FOXO1) [32]. Although in vitro or ex vivo glucose treatments have yet to be completed in skeletal muscle, protein and mRNA analysis of skeletal muscle tissue from NGT, IGT, and T2DM have shown that TXNIP expression increases as glucose tolerance decreases, indicating that elevated plasma glucose levels may be a potent stimulus to promote TXNIP gene expression in skeletal muscle as well [33].

Furthermore, free fatty acids can also augment the gene expression of TXNIP. C2C12 skeletal muscle cells treated with the long-chain saturated acid, palmitic acid (PA) increased ROS production and TXNIP expression in a dose-dependent manner. However, this effect was attenuated following co-incubation with Fenofibrate or N-Acetyl-L-cysteine (NAC), a ROS-scavenging compound [24]. In agreement, others have shown that an increase in ROS production following treatment with glucose or known oxidative stressors such as paraquat, ultraviolet (UV) radiation, or H2O2 is essential for the upregulation of TXNIP expression [32,34,35]. Although this explains the blunted gene expression response following treatment with the antioxidant NAC, Fenofibrate, can act as an exercise mimetic capable of activating the sirtuin 1 (SIRT1)/AMP-activated protein kinase (AMPK) axis. To tease out the specific mechanism, the investigators co-incubated cells with PA, Fenofibrate and inhibitors of its known downstream effectors, AMP-activated protein kinase (AMPK) and sirtuin 1 (SIRT1). Following the addition of compound C (AMPK inhibitor), Fenofibrate was no longer capable of downregulating TXNIP expression in response to PA treatment [24].

The role of AMPK activation in reducing TXNIP expression has been investigated further. Shaked, et al. [36] performed a dose-response curve with the exercise-mimetic, metformin, in glucose-treated rat hepatocytes. As metformin concentration increased, the ability for glucose to stimulate TXNIP gene expression concurrently decreased. Chromatin immunoprecipitation analysis revealed that AMPK activation following metformin treatment led to exclusion of ChREBP from the nucleus, preventing its binding to the promoter site of TXNIP. AMPK can alternatively affect TXNIP protein levels via post-translational mechanisms as well. Primary rat hepatocytes treated with AMPK agonists, 5-Aminomidazole-4-carboxamide ribonucleotide (AICAR) and phenformin, resulted in the phosphorylation of TXNIP at ser308, inducing a conformational change that increased TXNIP’s susceptibility to ubiquitination and proteasome degradation [14]. Interestingly, this post-translational event is a shared consequence between AMPK activation and insulin stimulation, alike. Recent investigation in 3T3L1 adipocytes has indicated that TXNIP is a substrate for protein kinase B (AKT) and upon insulin stimulation, AKT can phosphorylate TXNIP at ser308, and increase its rate of proteasome degradation, which is required for insulin-mediated glucose uptake [25]. However, individuals with insulin resistance have impaired insulin-mediated AKT activation [37], providing further insight into why TXNIP is overexpressed in skeletal muscle and adipose tissue of these individuals [33].

Although AMPK can be activated via pharmaceutical means, its physiological role is to sense and respond to the cellular energy state. Elevated [AMP], [ADP], and [NAD+], hypoxia, and glycogen depletion have been shown to activate AMPK which initiates glucose and fat uptake to increase substrate availability for ATP production. TXNIP has been shown to inhibit glucose uptake [33], therefore AMPK’s ability to increase its rate of degradation may be an indirect mechanism to improve intracellular glucose availability. Physiological stressors that have been shown to activate AMPK in vivo include...
caloric restriction and aerobic exercise [38-41]. Johnson, et al. [42] completed a 16-week caloric restriction intervention to understand the mechanisms that underpin the improvements in insulin sensitivity seen in obese subjects. Interestingly, TXNIP protein levels decreased because of the intervention, which was positively correlated with clamp-derived glucose disposal rate. Additionally, a recent global gene analysis was conducted in skeletal muscle biopsies taken from young lean, healthy males following nine-days bedrest and subsequent retraining period showed that TXNIP gene expression was markedly reduced following the four-week retraining period. Similar to caloric restriction, these reductions were strongly associated with improvements in insulin sensitivity [43]. Although this investigation provided some substantial insight, the effect of exercise on TXNIP remains largely unknown. In future analysis, it would be interesting to see if improvements in insulin sensitivity following exercise are related to changes in TXNIP protein expression in the skeletal muscle, and if so, how efficacious it would be to target TXNIP as a therapeutic intervention for individuals with insulin resistance and/or T2DM.

Conclusions and Future Directions

TXNIP has been of interest to many as it plays a direct role in regulating both hepatic and peripheral glucose metabolism. However, less is known about its own regulation and the effect that changes in its expression have on glucose metabolism in vivo. Aerobic exercise may present as an interesting model to explore TXNIP further as it activates signaling pathways which have been shown to alter TXNIP expression in vitro. Further, acute exercise has been shown to increase insulin sensitivity in the post-exercise state, but the mechanism remains largely unknown. In future analysis, it would be interesting to see if improvements in insulin sensitivity following exercise are related to changes in TXNIP protein expression in the skeletal muscle, and if so, how efficacious it would be to target TXNIP as a therapeutic intervention for individuals with insulin resistance and/or T2DM.

Disclosure Statement

The authors report no conflicts of interest.

Author Contributions

All authors contributed equally to this work.

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