International Journal of
Diabetes and Clinical Research

REVIEW ARTICLE

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

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-κB: 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; P300: Histone Acetyltransferase p300; P38 MAPK: P38 Mitogen-Activated Protein Kinase; FOXO1: Forkhead 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

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 antioxidants Thioredoxin-1 (TRX-1) and 2 (TRX-2) and inhibiting

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 Ubiqutin 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 hypocholesteremia 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 CO_2 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. Oslowski, 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 D$_3$-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 mediates 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 via gene silencing, that the effect was dependent on the recruitment of transcription factor, ChREPB and histone 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 TXNIIP 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 H$_2$O$_2$ 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-Aminoimidazole-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 protein expression and its intermolecular interactions have yet to be elucidated.

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.

References

1. Control CfD, Prevention: National diabetes statistics report, 2017. Atlanta, GA: Centers for Disease Control and Prevention, US Department of Health and Human Services 2017;

2. Forbes JM, Coughlan MT, Cooper ME (2008) Oxidative stress as a major culprit in kidney disease in diabetes. Diabetes 57: 1446-1454.

3. Huxley R, Barzi F, Woodward M (2006) Excess risk of fatal coronary heart disease associated with diabetes in men and women: meta-analysis of 37 prospective cohort studies. BMJ 332: 73-78.

4. Logroscino G, Kang JH, Grodstein F (2004) Prospective study of type 2 diabetes and cognitive decline in women aged 70-81 years. BMJ 328: 548.

5. Nooyens AC, Baan CA, Spijkerman AM, Verschuren WM (2010) Type 2 diabetes and cognitive decline in middle-aged men and women. Diabetes Care 33: 1964-1969.

6. DeFronzo RA, Tripathy D (2009) Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. Diabetes Care 32: S157-S163.

7. Chen KS, DeLuca HF (1994) Isolation and characterization of a novel cDNA from HL-60 cells treated with 1, 25-dihydroxyvitamin D-3. Biochim Biophys Acta 1219: 26-32.

8. Nishiyama A, Matsui M, Iwata S, Hirota K, Masutani H, et al. (1999) Identification of thioredoxin-binding protein-2/vitamin D3 up-regulated protein 1 as a negative regulator of thioredoxin function and expression. J Biol Chem 274: 21645-21650.

9. Patwari P, Higgins LJ, Chutkow WA, Yoshioka J, Lee RT (2006) The interaction of thioredoxin with Txnip evidence for formation of a mixed disulfide by disulfide exchange. J Biol Chem 281: 21884-21891.

10. Spindel ON, World C, Berk BC (2012) Thioredoxin interacting protein: Redox dependent and independent regulatory mechanisms. Antioxid Redox Signal 16: 587-596.

11. Luttrell L, Ferguson S, Daaka Y, Miller W, Maudsley S, et al. (1999) Beta-Arrestin-dependent formation of beta2 adrenergic receptor-Src protein kinase complexes. Science 283: 655-661.

12. Miller WE, McDonald PH, Cai SF, Field ME, Davis RJ, et al. (2001) Identification of a motif in the carboxyl terminus of β-arrestin2 responsible for activation of JNK3. J Biol Chem 276: 27770-27777.

13. Zhang P, Wang C, Gao K, Wang D, Mao J, et al. (2010) The ubiquitin ligase itch regulates apoptosis by targeting thioredoxin-interacting protein for ubiquitin-dependent degradation. J Biol Chem 285: 8869-8879.

14. Wu N, Zheng B, Shaywitz A, Dagon Y, Tower C, et al. (2013) AMPK-dependent degradation of TXNIP upon energy stress leads to enhanced glucose uptake via GLUT1. Mol Cell 49: 1167-1175.

15. Spindel ON, Yan C, Berk BC (2012) Thioredoxin-Interacting Protein (TXNIP) Mediates Nuclear-to-Plasma Membrane Communication. Atherosclerosis.

16. Shin D, Jeon J-H, Jeong M, Suh H-W, Kim S, et al. (2008) VDUP1 mediates nuclear export of HIF1α via CRM1-dependent pathway. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research 1783: 838-848.

17. Bodnar JS, Chatterjee A, Castellani LW, Ross DA, Ohmen J, et al. (2002) Positional cloning of the combined hyperlipidemia gene Hyplip1. Nat Genet 30: 110-116.

18. Chutkow WA, Birkenfeld AL, Brown JD, Lee H-Y, Frederick DW, et al. (2010) Deletion of the alpha-arrestin protein Txnip in mice promotes adiposity and adipogenesis while preserving insulin sensitivity. Diabetes 59: 1424-1434.

19. Chutkow WA, Patwari P, Yoshioka J, Lee RT (2008) Thioredoxin-interacting protein (Txnip) is a critical regulator of hepatic glucose production. J Biol Chem 283: 2397-2406.

20. DeBalsi KL, Wong KE, Koves TR, Slentz DH, Seiler SE, et al. (2014) Targeted metabolomics connects thioredoxin-interacting protein (TXNIP) to mitochondrial fuel selection and regulation of specific oxidoreductase enzymes in skeletal muscle. J Biol Chem 289: 8106-8120.

21. Oslowski CM, Hara T, O'Sullivan-Murphy B, Kanekura K, Lu S, et al. (2012) Thioredoxin-interacting protein mediates ER stress-induced β cell death through initiation of the inflammasome. Cell Metab 16: 265-273.

22. Liu Y, Lian K, Zhang L, Wang R, Yi F, et al. (2014) TX-NIP mediates NLRP3 inflammasome activation in cardiac microvascular endothelial cells as a novel mechanism in myocardial ischemia/reperfusion injury. Basic Res Cardiol 109: 415.

23. Vandanmagsar B, Youm Y-H, Ravussin A, Galgani JE, Stadler K, et al. (2011) The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nat Med 17: 179-188.

24. Mandala A, Das N, Bhattacharjee S, Mukherjee B, Mukhopadhyay S, et al. (2016) Thioredoxin interacting protein mediates lipid-induced impairment of glucose uptake in skeletal muscle. Biochem Biophys Res Commun 479: 933-939.

25. Waldhart AN, Dykstra H, Peck AS, Boguslawski EA, Madaj ZB, et al. (2017) Phosphorylation of TXNIP by AKT mediates acute influx of glucose in response to insulin. Cell Rep 19: 2005-2013.

26. Devi TS, Hosoya K-I, Terasaki T, Singh LP (2013) Critical role of TXNIP in oxidative stress, DNA damage and retinal pericyte apoptosis under high glucose: Implications for diabetic retinopathy. Exp Cell Res 319: 1001-1012.

27. Kim GS, Jung JE, Narasimhan P, Sakata H, Chan PH (2012) Induction of thioredoxin-interacting protein is mediated by oxidative stress, calcium, and glucose after brain injury in mice. Neurobiol Dis 46: 440-449.

28. Cha-Molstad H, Saxena G, Chen J, Shalev A (2009) Glucose-stimulated expression of Txnip is mediated by carbohydrate response element-binding protein, p300, and histone H4 acetylation in pancreatic beta cells. J Biol Chem 284: 16898-16905.

29. Chen JL, Merl D, Peterson CW, Wu J, Liu PY, et al. (2010) Lactic acidosis triggers starvation response with paradoxical induction of TXNIP through MondoA. PLoS Genet 6: e1001093.

30. Xu G, Chen J, Jing G, Shalev A (2012) Preventing β-cell loss and diabetes with calcium channel blockers. Diabetes 61: 848-856.

31. Kibbe C, Chen J, Xu G, Jing G, Shalev A (2013) FOXO1 competes with carbohydrate response element-binding protein (ChREBP) and inhibits thioredoxin-interacting protein (TXNIP) transcription in pancreatic beta cells. J Biol Chem 288: 23194-23202.

32. Li X, Rong Y, Zhang M, Wang XL, LeMaire SA, et al. (2009) Up-regulation of thioredoxin interacting protein (Txnip) by p38 MAPK and FOXO1 contributes to the impaired thioredoxin activity and increased ROS in glucose-treated endothelial cells. Biochem Biophys Res Commun 381: 660-665.

33. Parikh H, Carlsson E, Chutkow WA, Johansson LE, Storgaard H, et al. (2007) TXNIP regulates peripheral glucose metabolism in humans. PLoS Med 4: e158.

34. Fang S, Jin Y, Zheng H, Yan J, Cui Y, et al. (2011) High glucose condition upregulated Txnip expression level in rat mesangial cells through ROS/MEK/MAPK pathway. Mol Cell Biochem 347: 175-182.

35. Junn E, Han SH, Im JY, Yang Y, Cho EW, et al. (2000) Vitamin D3 up-regulated protein 1 mediates oxidative stress via suppressing the thioredoxin function. J Immunol 164: 6287-6295.

36. Shaked M, Ketzinel-Gilad M, Cerasi E, Kaiser N, Leibowitz G (2011) AMP-activated protein kinase (AMPK) mediates nutrient regulation of thioredoxin-interacting protein (TXNIP) in pancreatic beta-cells. PLoS One 6: e28804.

37. Kim YB, Nikoulina SE, Ciaraldi TP, Henry RR, Kahn BB (1999) Normal insulin-dependent activation of Akt/protein kinase B, with diminished activation of phosphoinositide 3-kinase, in muscle in type 2 diabetes. J Clin Invest 104: 733-741.

38. Garcia-Prieto C, Pulido-Olmo H, Ruiz-Hurtado G, Gil-Ortega M, Aranguez I, et al. (2015) Mild caloric restriction reduces blood pressure and activates endothelial AMPK-PI3K-Akt-eNOS pathway in obese Zucker rats. Vascul Pharmacol 65-66: 3-12.

39. Civitarese AE, Carling S, Heilbronn LK, Hulver MH, Ukropcova B, et al. (2007) Calorie restriction increases muscle mitochondrial biogenesis in healthy humans. PLoS Med 4: e76.

40. Musi N, Fujii N, Hirshman MF, Ekberg I, Fröberg S, et al. (2001) AMP-activated protein kinase (AMPK) is activated in muscle of subjects with type 2 diabetes during exercise. Diabetes 50: 921-927.

41. Gibala MJ, McGee SL, Garnham AP, Howlett KF, Snow RJ, et al. (2009) Brief intense interval exercise activates AMPK and p38 MAPK signaling and increases the expression of PGC-1alpha in human skeletal muscle. J Appl Physiol 106: 929-934.

42. Johnson ML, Distelmaier K, Lanza IR, Irving BA, Robinson MM, et al. (2016) Mechanism by which caloric restriction improves insulin sensitivity in sedentary obese adults. Diabetes 65: 74-84.

43. Alibegovic AC, Sonne MP, Højbjerre L, Bork-Jensen J, Jacobsen S, et al. (2010) Insulin resistance induced by physical inactivity is associated with multiple transcriptional changes in skeletal muscle in young men. Am J Physiol Endocrinol Metab 299: E752-E763.