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REVIEW

High-fat load: mechanism(s) of insulin resistance in skeletal muscle

DS Lark[1,2], KH Fisher-Wellman[1,2] and PD Neufer[1,2,3]

Skeletal muscle from sedentary obese patients is characterized by depressed electron transport activity, reduced expression of genes required for oxidative metabolism, altered mitochondrial morphology and lower overall mitochondrial content. These findings imply that obesity, or more likely the metabolic imbalance that causes obesity, leads to a progressive decline in mitochondrial function, eventually culminating in mitochondrial dissolution or mitoptosis. A decrease in the sensitivity of skeletal muscle to insulin represents one of the earliest maladies associated with high dietary fat intake and weight gain. Considerable evidence has accumulated to suggest that the cytosolic ectopic accumulation of fatty acid metabolites, including diacylglycerol and ceramides, underlies the development of insulin resistance in skeletal muscle. However, an alternative mechanism has recently been evolving, which places the etiology of insulin resistance in the context of cellular/mitochondrial bioenergetics and redox systems biology. Overnutrition, particularly from high-fat diets, generates fuel overload within the mitochondria, resulting in the accumulation of partially oxidized acylcarnitines, increased mitochondrial hydrogen peroxide (H_2O_2) emission and a shift to a more oxidized intracellular redox environment. Blocking H_2O_2 emission prevents the shift in redox environment and preserves insulin sensitivity, providing evidence that the mitochondrial respiratory system is able to sense and respond to cellular metabolic imbalance. Mitochondrial H_2O_2 emission is a major regulator of protein redox state, as well as the overall cellular redox environment, raising the intriguing possibility that elevated H_2O_2 emission from nutrient overload may represent the underlying basis for the development of insulin resistance due to disruption of normal redox control mechanisms regulating protein function, including the insulin signaling and glucose transport processes.

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INTRODUCTION

Deciphering the underlying mechanism(s) responsible for the development of insulin resistance in peripheral tissues is one of the cornerstones to understanding the etiology of type 2 diabetes. A gradual decrease in the sensitivity of skeletal muscle to insulin is considered a primary event in the disease process and, as such, likely holds the key to devising more effective prevention and treatment strategies. By virtue of its high percentage of total body mass and sensitivity to insulin, skeletal muscle accounts for the vast majority ($\sim$80%) of glucose disposal.[1] The control of glucose uptake is distributed across delivery, transport and phosphorylation, any one of which may be rate limiting depending on the physiological circumstances.[2] Insulin increases glucose delivery via relaxation of resistance vessels to increase total blood flow and relaxation of precapillary arterioles to increase microvascular surface area perfusion within muscle, thereby increasing the trans-endothelial transport of insulin and glucose.[3] Although there is some evidence that insulin resistance induced by a high-fat diet compromises glucose delivery,[4] more research is needed to fully define this potential mechanism of action. The glucose transport process adds further potential control points beginning with insulin binding to and activating its receptor, and progressing through activation of downstream intracellular signaling events leading to the translocation (that is, budding, transport, tethering, docking, fusion and endocytosis) and activation of the GLUT4 transporter protein.[5,6] There is compelling evidence that phosphorylation of glucose, the third potential control point, is the rate-limiting step for glucose uptake in response to insulin or exercise in skeletal muscle with normal insulin sensitivity;[7,8] however, the functional barrier appears to shift to transport in the insulin-resistant state.[7,9,10] GLUT4 translocation process does not appear to be defective in insulin-resistant muscle, as both contraction and hypoxia, which utilize a signaling pathway different from insulin, stimulate GLUT4 translocation and glucose uptake normally.[11,12] Thus, most of the research since the mid-1990s has focused on deciphering the mechanism(s) by which the insulin signaling pathway is inhibited by a high-fat diet. The present review provides a brief overview of this research and then presents an alternative hypothesis to link mitochondrial H_2O_2 emission with insulin resistance during nutrient overload.

INTRAMYOCELLULAR FAT ACCUMULATION

Triacylglycerol

Skeletal muscle of obese and diabetic individuals is characterized by a greater size and number of lipid droplets.[13] Once assumed to be relatively inert, lipid droplets are now known to be coated with phospholipids and a variety of proteins, to exist in a regulated equilibrium of triglyceride synthesis and degradation and to participate in other cellular processes including vesicle trafficking and cell signaling.[14–16] One possibility is that fat deposition in tissues not specialized for fat storage can promote lipotoxicity.[17]

[1]East Carolina Diabetes and Obesity Institute, East Carolina University, Greenville, NC, USA; [2]Department of Kinesiology, East Carolina University, Greenville, NC, USA and [3]Department of Physiology, Brody School of Medicine, East Carolina University, Greenville, NC, USA. Correspondence: Dr PD Neufer, Department of Physiology, Brody School of Medicine, 6N98, East Carolina University, Greenville, NC 27834, USA.
E-mail: neuferp@ecu.edu
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Numerous initial studies in both humans and rodents reported a strong correlation between intramuscular triglyceride content and insulin resistance.[18–23] However, endurance-trained athletes also have high intramuscular triglyceride content, as well as high insulin sensitivity,[24] indicating that triglyceride accumulation *per se* is not an underlying cause of insulin resistance. In fact, mice with skeletal muscle-specific overexpression of diacylglycerol acyltransferase (DGAT1), the final enzyme in the TAG synthesis pathway, are protected from high-fat diet-induced insulin resistance despite higher muscle TAG content.[25] Nevertheless, given the dynamic interplay between triglyceride synthesis and storage, it has recently been suggested that a mismatch between these processes could give rise to potential insulin-desensitizing lipid intermediates.[14,15]

Diacylglycerol (DAG) and long-chain acyl-coAs (LCACoAs)

Further clues as to the mechanism by which fat accumulation in muscle may lead to insulin resistance have come from studies of the signaling pathway acting downstream of the insulin receptor. Under normal circumstances, activation of the insulin receptor tyrosine kinase and subsequent tyrosine phosphorylation of the insulin receptor substrate (IRS) docking proteins leads to the recruitment and activation of phosphoinositide 3-kinase (PI3K). The major substrate for PI3K is the membrane lipid phosphatidylinositol-4,5-bisphosphate (PI(4,5,)P_2), which is phosphorylated to produce phosphatidylinositol-3,4,5-trisphosphate (PI(3,4,5,)P_3). The rise in PI(3,4,5,)P_3 provides a lipid-based platform that attracts downstream pleckstrin homology domain-containing signaling proteins, including the serine/threonine kinases phosphoinositide-dependent protein kinase-1 (PDK1) and Akt.[6] PDK1 phosphorylates Akt and atypical protein kinase C isoforms λ and ζ (aPKC-λ/ζ). Both Akt and aPKC-λ/ζ have been linked to insulin-stimulated GLUT4 translocation and glucose uptake.

In addition to tyrosine residues, phosphorylation of IRS proteins on serine residues has emerged as a major control point for the insulin signaling pathway. Of the $\sim$70 serine residues at potential consensus phosphorylation sites, more than 20 have been identified by proteomics to be phosphorylated by insulin,[26] and more than a dozen of these sites are subject to reversible phosphorylation by at least 16 different kinases, including 2 of the novel PKC isoforms (PKC δ and θ).[27] Given that both LCACoAs and DAG are well-established activators of PKCs,[28] much attention has been given to the possibility that novel PKCs may mediate the serine phosphorylation and inactivation of IRS proteins. LCACoAs and DAGs both accumulate in muscle with lipid infusion or high-fat feeding[29] and are associated with membrane translocation and activation of PKC-θ, increased IRS-1 serine phosphorylation and decreased insulin-stimulated IRS-1 tyrosine phosphorylation, IRS-1-associated PI3K activity and muscle glucose uptake.[30–32] In cultured myocytes, expression of active PKC-θ increases phosphorylation of IRS-1 at Ser^{1101} and blocks insulin-stimulated IRS-1 tyrosine phosphorylation and activation of Akt, whereas mutation of Ser^{1101} to alanine renders IRS-1 insensitive to PKC-θ and restores downstream insulin signaling.[33] Similarly, PKC-θ kinase activity has recently been shown to mediate the serine phosphorylation and inactivation of PDK1 in mouse embryonic fibroblast cells exposed to palmitate.[34] Finally, and most compelling, PKC-θ knockout mice were found to be completely protected against lipid infusion-induced disruptions in insulin signaling and muscle glucose uptake.[35]

Evidence specifically linking PKC-θ to insulin resistance, however, has also been challenged. Mice expressing a kinase-dead, dominant-negative mutant form of PKC-θ specifically in skeletal muscle, rather than being protected from fat-induced insulin resistance, have impaired insulin signaling in muscle by 4 months of age and develop insulin resistance and obesity on a chow diet by 6 to 7 months of age.[36] PKC-θ may mediate its effects indirectly via other signaling kinases, such as PDK1, JNK and/or IKK, or may be compensated for by the highly homologous PKCδ.[33] Curiously, no studies have been conducted on either PKC-θ knockout or muscle-specific, dominant-negative mice on a high-fat diet, the more physiologically relevant model of insulin resistance, nor have mice expressing a constitutively active or inducible PKC-θ specifically in skeletal muscle been generated.

Sphingosines and ceramides

In addition to LCACoA and DAG, the accumulation of the sphingolipid intermediate ceramide has also been suggested to have a role in lipid-induced insulin resistance.[37,38] Intracellular accumulation of ceramide occurs in response to numerous cellular stressors (for example, cytokines, hypoxia and ROS) via the stress-induced activation of sphingomyelinases/glucosidases and/or the suppression of ceramide clearance.[39] High-fat diets and lipid infusion have been shown to increase intracellular ceramide, impair insulin signaling and decrease glucose uptake in skeletal muscle of rodents[40] and humans,[41] as well as in cultured myotubes.[42] Inhibition of *de novo* ceramide synthesis prevents the development of muscle insulin resistance in response to lipid infusion and in various diet-induced obesity models,[43–45] providing fairly compelling evidence that ceramide accumulation may be a key factor in the etiology of insulin resistance. However, no increase in ceramide content was detected in at least two other studies using lipid infusion to induce insulin resistance in rats or humans,[46,47] and no differences in ceramide levels were found in the skeletal muscle from type 2 diabetic versus healthy individuals.[48] In addition, at least one of the inhibitors used to inhibit ceramide (myriocin) has also been shown to increase energy expenditure in treated animals,[44] potentially complicating the interpretation of these studies.[49] Thus, it appears that further work is required to determine whether ceramide accumulation is necessary and/or sufficient for the induction of diet-induced insulin resistance in skeletal muscle.

INFLAMMATION

Numerous lines of evidence have established a link between elevated systemic, as well as tissue-derived, inflammation with the development of insulin resistance (for review see Heilbronn and Campbell[50] and Schenk *et al.*[51]). As such, elevations in proinflammatory cytokines (tumor necrosis factor-α interleukin-6) and C-reactive protein have repeatedly been observed in the plasma, as well as within peripheral insulin target tissues (for example, liver, adipose tissue and skeletal muscle), of both animal (high-fat diet) and human (obesity and type 2 diabetes) models of insulin resistance (for review see de Luca and Olefsky[52] and Shoelson *et al.*[53]). Regardless of the origin/site of inflammation, the effectors of insulin resistance within peripheral tissues in the context of high-fat diet-induced inflammation are believed to involve hyperactivation of stress-sensitive Ser/Thr kinases, such as JNK[54] and IKKβ,[55] due in large part to increased signaling through the JNK/activator protein 1 (AP1) and IKK/NF-κB pathways. Overactivation of these signaling pathways and their associated Ser/Thr kinases is expected to induce reductions in insulin sensitivity through a similar mechanism as that described previously for intracellular lipid accumulation (for example, inhibitory serine phosphorylation of the insulin receptor:IRS1 axis).[27]

At present, it would appear that the eventual manifestation of an insulin-resistant phenotype likely results from, or is exacerbated by, the summation of proinflammatory responses that occur systemically,[56] as well as within each peripheral tissue bed (adipose,[57,58] skeletal muscle[59,60]) as a consequence of high-fat diet exposure. In other words, activation of proinflammatory cascades seems to be a general response within all cells under conditions of nutrient overload. Although the association between

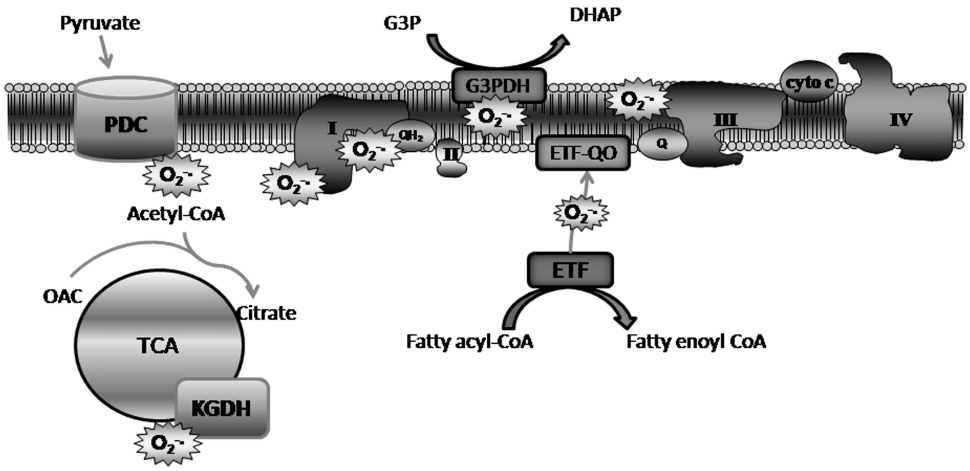

Figure 1. Potential sites of mitochondrial superoxide ($O_2^{\bullet -}$) generation. Mitochondrial-derived $O_2^{\bullet -}$ can arise from any one of seven known sites of electron leak, including the flavin mononucleotide- and ubiquinone-binding site within complex I, the quinone at centre 'o' within complex III, the quinone-binding site within G3PDC, the ETF-QOR complex and the matrix dehydrogenase enzyme complexes PDC and KGDH. Cyto *c*, cytochrome *c*; OAC, oxaloacetate; QH_2, ubiquinol; Q, ubiquinone.

inflammation and high-fat diet-mediated insulin resistance appears to be well established, a central question remains: what is the mechanism by which excessive nutrient/lipid supply serves to activate proinflammatory cascades within peripheral tissues? Suggested mechanisms have included the following: (1) micro-hypoxic conditions within engorged adipocyte depots,[61] (2) adipocyte cell death/necrosis,[62] (3) endoplasmic reticulum stress[63] and (4) activation of cytotoxic T cells following ligation of T-cell receptors via specific fatty acid species.[58] An additional potential unifying mechanism involves the induction of intrinsic proinflammatory cascades within peripheral cells as a consequence of elevated H_2O_2 production.[64–66] That is, activation of the aforementioned proinflammatory pathways (JNK/AP1 and IKK/NF-κB) is also possible within peripheral tissues following an oxidative shift in the cellular redox environment, without any need for macrophage activation/infiltration.[67] This later hypothesis is intriguing given that mitochondrial-derived hydrogen peroxide (H_2O_2), a major regulator of the cellular redox environment, has recently been linked to the etiology of diet-induced insulin resistance.[68–70] To understand the mechanisms by which mitochondrial-derived ROS are believed to interact with and ultimately impair cellular signaling in response to insulin, a brief discussion of the factors governing mitochondrial H_2O_2 generation is necessary.

MITOCHONDRIAL H_2O_2 EMISSION, THE REDOX ENVIRONMENT AND INSULIN RESISTANCE

H_2O_2 is the nonradical dismutation product of superoxide ($O_2^{\bullet -}$), the short-lived parent molecule of all ROS. Although there are numerous sources of H_2O_2, mitochondrial-derived H_2O_2 is considered the major source in biological systems.[71] Mitochondrial H_2O_2 emission reflects the balance between the rate of $O_2^{\bullet -}$/H_2O_2 formation and scavenging within the matrix, with emitted H_2O_2 serving to regulate the intracellular redox environment in favor of greater reducing (↓ H_2O_2) or oxidizing (↑ H_2O_2) conditions.[72] Currently, identified sites of electron leak within the respiratory chain include the flavin mononucleotide (site IF) and ubiquinone-binding site (site IQ) within complex I, the quinone at centre 'o' within complex III (site IIIQo), the quinone-binding site within glycerol-3-phosphate dehydrogenase (G3PDH) and electron-transferring flavoprotein Q oxidoreductase (ETF-QOR).[73] Additional nonrespiratory chain sites include two matrix dehydrogenase enzyme complexes, pyruvate dehydrogenase (PDH) and α-ketoglutarate dehydrogenase (KGDH)[74] (Figure 1).

Regardless of the site, mitochondrial H_2O_2 is strongly dependent on overall metabolic balance, as maximal rates of electron leak occur under elevated reducing conditions (↑ NAD(P)H/NAD(P)+, QH_2/Q), in which mitochondrial membrane potential ($\Delta\psi$) is highly negative and the overall demand for ATP synthesis is low.[75,76] These criteria are met under conditions that mimic state 4 respiration, where respiring mitochondria are fully saturated with substrate, yet not actively phosphorylating ADP.[77] Although it is unlikely that cells *in vivo* are ever truly engaged in state 4 respiration, near state 4 conditions likely occur during periods of nutrient overload combined with minimal ATP demand (that is, high caloric intake combined with a sedentary lifestyle). These conditions would be expected to elevate the reducing pressure within the respiratory chain, accelerate mitochondrial $O_2^{\bullet -}$ generation/H_2O_2 emission and trigger an oxidative shift in the redox environment. In support of this notion, high dietary fat intake generates an increase in partially oxidized lipid intermediates indicative of mitochondrial overload[78] and decreases the GSH/GSSG ratio in muscle, indicative of a shift in the intracellular redox environment to a more oxidized state.[68] This appears to be mediated, at least in part, by a remarkable increase in the propensity for mitochondrial H_2O_2 emission, implying some type of alteration in the governance of H_2O_2 production/emission in response to the lipid overload.[68] Treatment of high-fat-fed rodents with SS31 (mitochondrial-targeted small antioxidant peptide), as well as the transgenic expression of the human catalase gene within muscle mitochondria (*mCAT*), completely blocked the development of insulin resistance, as well as the associated increase in H_2O_2-emitting potential and oxidative shift in the redox environment. Interestingly, mCAT mice fed a standard chow diet exhibit improved skeletal muscle insulin sensitivity, as well as reduced H_2O_2-emitting potential, compared with chow-fed WT mice.[68]

Collectively, these findings suggest that the degree of skeletal muscle insulin sensitivity within a cell may rely on the degree of reduction or oxidation within the intracellular redox environment. In this context, H_2O_2 emission by the mitochondria and the resulting oxidative shift in the cellular redox environment during nutrient overload is viewed as a metabolic feedback sensor to decrease insulin sensitivity.[79] The mechanism(s) by which H_2O_2-mediated redox control regulates insulin sensitivity is unknown. Given the sensitivity of cellular phosphatases to redox state,[80] it has recently been suggested that elevated mitochondrial H_2O_2 emission may lead to a decrease in the normally dominant global

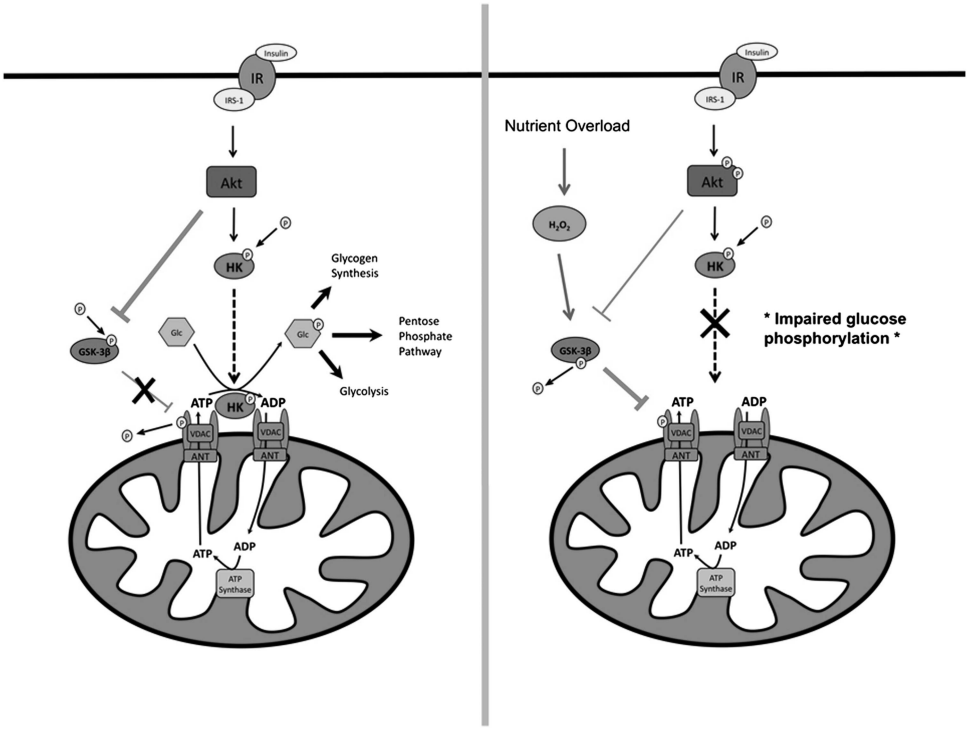

Figure 2. Effects of insulin and H_2O_2 on HK association with mitochondria. Left panel: HK association with mitochondria is promoted by insulin-mediated Akt phosphorylation of HK. Concurrently, Akt phosphorylation of GSK-3β relieves tonic phosphorylation of VDAC, resulting in increased HK/VDAC-binding affinity. When bound to mitochondria, HK is thought to gain a bioenergetic advantage via coupling with oxidative phosphorylation by virtue of the fusion of the outer and inner mitochondrial membranes through interaction between VDAC/ANT. Right panel: exogenous H_2O_2 dissociates HK from the mitochondria in cardiomyocytes through an unknown mechanism. GSK-3β activity is increased by exogenous H_2O_2, which is hypothesized to decrease binding of HK to VDAC, leading to HK dissociation from mitochondria, which is proposed to contribute to the etiology of diet-induced insulin resistance by impairing glucose phosphorylation in skeletal muscle.

phosphatase tone in cells, increasing the susceptibility of insulin signaling proteins to inhibitory serine/threonine phosphorylation by stress-sensitive kinases (that is, JNK/AP1 and IKK/NF-κB; for review see Fisher–Wellman and Neufer[79]). Another possibility is that elevated H_2O_2 emission may directly target a key component of the glucose uptake process itself.

ALTERATIONS IN HEXOKINASE (HK) DURING NUTRIENT OVERLOAD

Glucose phosphorylation is an integral step for insulin-stimulated glucose uptake and has been shown to be functionally impaired during nutrient overload induced by a high-fat diet[81,82] and in association with type 2 diabetes.[83] Glucose phosphorylation is catalyzed by HK, and gene and protein expression of HKII, the predominant isoform found in skeletal muscle, is increased in response to insulin[84] but depressed in patients with type 2 diabetes.[85,86] HKI and HKII can also bind to the mitochondrial outer membrane through interactions with mitochondrial porin in skeletal muscle.[87] Intriguingly, overexpression of HKII in insulin-resistant mice fed a high-fat diet improves exercise-stimulated, but not insulin-stimulated, glucose uptake.[7] This presents a quandary: why does not increasing glucose phosphorylating capacity (via HKII overexpression) improve glucose uptake if glucose phosphorylation is rate limiting? One possibility is that glucose phosphorylation is not solely a function of HKII content, but also dependent upon the subcellular localization of HK with mitochondria. When bound to mitochondria in skeletal muscle, HK displays greater sensitivity for ATP derived from mitochondria than exogenous ATP,[88] suggesting that HK association with mitochondria provides a bioenergetic advantage to glucose phosphorylation. Insulin promotes HKII association with the mitochondrial outer membrane in both rodent[89] and human[90] striated muscle, and may do so via Akt in two ways: (1) direct phosphorylation of HKII[91] and (2) inhibition of glycogen synthase kinase-3β (GSK-3β),[92] a basally active negative regulator of glycogen synthesis. Inhibition of GSK-3β decreases phosphorylation tone on VDAC, which increases the binding affinity between VDAC and HK.[91] GSK-3β activity is increased during nutrient overload[93] and oxidative stress,[94] whereas muscle-specific overexpression of GSK-3β is casually linked to insulin resistance.[95] In addition, exogenous H_2O_2 has been shown to dissociate HKII from mitochondria in cultured cardiomyocytes,[96] providing a potential direct link between mitochondrial/cellular redox control and HK association with mitochondria (Figure 2). Whether elevated mitochondrial H_2O_2 emission leads to dissociation of HK from mitochondria and thereby contributes to high-fat diet-induced insulin resistance awaits further investigation.

CONCLUDING REMARKS

Research conducted over the last several decades has firmly established a link between positive metabolic balance and the development of metabolic disease (for example, insulin resistance, type 2 diabetes). However, deciphering the mechanism(s) by which chronic excess nutrient supply actually interacts with and impairs insulin signaling, ultimately leading to the clinical manifestation of insulin resistance, has proven difficult. Evidence is accumulating that adaptations within the mitochondria, either in 'response to' or 'as a consequence of' excessive nutrients, likely underlie this process. Further elucidation of the complex relationships between metabolic balance, H_2O_2 emission and cellular

redox environment will be necessary to allow for the eventual design/development of pharmacological/dietary interventions designed to restore/prevent metabolic disease.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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