CALL FOR PAPERS | Mitochondria in Cardiovascular Physiology and Disease

Redox regulation of insulin sensitivity due to enhanced fatty acid utilization in the mitochondria

Paul M. Rindler,1 Clair L. Crewe,1,2 Jolyn Fernandes,1,2 Michael Kinter,1,3 and Luke I. Szweda1,2,3

1Free Radical Biology and Aging Research Program, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma; 2Department of Biochemistry and Molecular Biology, University of Oklahoma Health Science Center, Oklahoma City, Oklahoma; and 3Department of Geriatric Medicine, Reynolds Center on Aging, University of Oklahoma Health Science Center, Oklahoma City, Oklahoma

Submitted 24 October 2012; accepted in final form 17 June 2013

Rindler PM, Crewe CL, Fernandes J, Kinter M, Szweda LI. Redox regulation of insulin sensitivity due to enhanced fatty acid utilization in the mitochondria. Am J Physiol Heart Circ Physiol 305: H634–H643, 2013. First published June 21, 2013; doi:10.1152/ajpheart.00799.2012.—Obesity enhances the risk for the development of type 2 diabetes and cardiovascular disease. Loss in insulin sensitivity and diminished ability of muscle to take up and use glucose are characteristics of type 2 diabetes. Paradoxically, regulatory mechanisms that promote utilization of fatty acids appear to initiate diet-induced insulin insensitivity. In this review, we discuss recent findings implicating increased mitochondrial production of the prooxidant H2O2 due to enhanced utilization of fatty acids, as a signal to diminish reliance on glucose and its metabolites for energy. In the short term, the ability to preferentially use fatty acids may be beneficial, promoting a metabolic shift that ensures use of available fat by skeletal muscle and heart while preventing intracellular glucose accumulation and toxicity. However, with prolonged consumption of high dietary fat and ensuing obesity, the near exclusive dependence on fatty acid oxidation for production of energy by the mitochondria drives insulin resistance, diabetes, and cardiovascular disease.

mitochondria; metabolic flexibility; redox signaling; insulin signaling; obesity

Introduction

The rising incidence of obesity has rapidly escalated into an imminent and costly threat to public health. In the United States, the Centers for Disease Control and Prevention report that the rate of obesity has risen threefold over the past thirty years with annual medical spending now exceeding $100 billion for obesity-related disorders (31). Obesity is the accumulation of excess body fat that occurs when caloric intake exceeds energy demand for extended lengths of time. Ironically, the ability to store energy as fat is a survival advantage when humans endure times of food scarcity. However, today’s constant availability of food, combined with a decline in physical activity, has turned this survival advantage into a health problem in which increasing body mass index has been linked to increased mortality (98). Obesity promotes conditions that increase risk of heart failure, such as hypertension and atherosclerosis, and is itself an independent risk factor for heart disease (1, 48, 51, 58). Moreover, obesity is also a major predictor for the development of type 2 diabetes and subsequent diabetic cardiomyopathy (12).

One hypothesis that requires further investigation is that excess caloric intake and accompanying obesity induce a state of oxidative stress that damages affected tissues and negatively impacts critical cellular and physiological processes. The goal of this review is to summarize the role of mitochondria in diet- and obesity-induced oxidative stress and the physiological and pathophysiological consequences. While our primary interest is the cardiomyocyte, information available on the heart is limited. As such, data from multiple tissues are discussed while highlighting what is known in cardiomyocytes. High dietary fat and obesity increase the supply of circulating free fatty acids and, subsequently, the reliance on fat for energy production by various tissues, including heart and mixed skeletal muscle, in particular slow twitch fibers which are metabolically similar to cardiac muscle (18, 58, 65, 87, 106). Evidence is presented indicating that mitochondria produce more hydrogen peroxide (H2O2) during the oxidation of fatty acids versus glycolytic metabolites. Furthermore, this difference in H2O2 production...
initiates a signal transduction cascade that serves to adjust metabolism based on dietary composition. Alterations in mitochondrial metabolism and \( \text{H}_2\text{O}_2 \)-induced reductions in insulin signaling may be considered beneficial in the short term. However, these regulatory mechanisms precipitate disease with chronic consumption of excessive calories that promote obesity.

**Obesity and Oxidative Stress**

Obesity in humans and a number of experimental models are associated with oxidative stress. Oxidative stress arises from an imbalance between the production and removal of free radicals and prooxidants. These reactive species are capable of oxidizing and, in many cases, damaging a variety of macromolecules including DNA, protein, lipid, and carbohydrate (8, 21, 26, 38). Given the relatively short half-life of most free radicals and prooxidants, the assessment of oxidative stress is often based on the measurement of oxidation products and the status of various antioxidants and redox couples.

Obese humans have elevated markers of oxidative stress in plasma and multiple tissues compared with lean healthy controls (95). Plasma obtained from obese subjects displays increased levels of 8-hydroxyguanosine and thiobarbituric acid reactive substances, markers of oxidized DNA and lipids, respectively. Increased lipid peroxidation products have been found in skeletal muscle and urine of obese individuals (95). More recently, elevated levels of oxidized proteins have been observed in subcutaneous adipose tissue (33, 69). The contribution of obesity to increased oxidative stress in the human heart has been difficult to assess given the limited amount of tissue available for study. However, a recent study revealed that right atrial cardiomyocytes obtained from non-diabetic obese men undergoing coronary artery bypass grafting display increased levels of DNA and protein oxidation products as well as increased apoptotic markers compared with normal weight patients (66). These data demonstrate that obesity in humans is accompanied by increased measures of oxidative stress within multiple tissues, including the heart.

Similar to humans, increased markers of oxidative damage have been observed in tissues of rodents in both genetic and diet-induced models of obesity. For example, in the mutant mouse strain KKAy, which has a mutation in the *Raly* gene leading to spontaneous development of obesity and type 2 diabetes, the animals have higher levels of \( \text{H}_2\text{O}_2 \) and lipid peroxidation products in both plasma and adipose tissue (35). In the leukocyte deficient \( \text{ob/ob} \) mice, which also develop obesity and type 2 diabetes, increased concentrations of lipid peroxidation products are seen in both plasma and skeletal muscle (78). Elevated protein and lipid oxidation products in adipose tissue, skeletal muscle, and/or liver have also been seen in mouse (39, 42) and rat (62) models of diet-induced obesity. Measures of protein glycation, a form of oxidative damage that can occur at high concentrations of sugars (34, 38), have been shown to increase in serum and adipose tissue of mice or rats fed high-fat diets (53, 79, 90). In the heart, \( \text{ob/ob} \) mice have decreased levels of reduced glutathione (80), and mice fed a high-fat diet have increased levels of protein oxidation products (96). These observations indicate that high dietary fat and obesity induce a general state of oxidative stress and have often led to the conclusion that oxidative damage contributes to accompanying pathophysiological events including cardiovascular disease. Despite frequently inferred relationships, however, evidence that free radical production and oxidative damage are causally linked to specific functional changes is lacking. In addition, oxidative stress does not in itself imply oxidative damage in that certain free radicals and prooxidants are capable of acting as signaling molecules (63, 73, 94).

Comprehensive assessment of the various mechanisms by which obesity induces oxidative stress could shed light on the physiological and pathophysiological consequences.

**Sources of Obesity Induced Oxidative Stress**

Mechanisms by which high dietary fat and obesity induce increases in the production of free radical species include infiltration of macrophages into adipose tissue, release of proinflammatory adipokines, elevated NADPH oxidase (Nox) expression/activity, and enhanced generation by mitochondria (27, 32, 68, 95). Given the central role of mitochondria in metabolic homeostasis, mitochondrial \( \text{H}_2\text{O}_2 \) production may represent a critical link between metabolic changes induced by high dietary fat and subsequent physiological and pathophysiological consequences of obesity. Reducing equivalents generated from the oxidation of energetic substrates donate electrons to the electron transport chain (ETC). These electrons normally pass between the ETC complexes within the mitochondrial inner membrane from high to low reductive potential. This energetically favorable process is coupled to the transfer of protons from the matrix to the inner membrane space to create an electrochemical gradient used by ATP synthase to generate ATP. Oxygen serves as the final electron acceptor with the four electron reduction to \( \text{H}_2\text{O} \). However, a small fraction of the electrons that pass through the ETC induce the incomplete one electron reduction of oxygen to form superoxide anion. Superoxide anion is then rapidly converted to \( \text{H}_2\text{O}_2 \) by superoxide dismutase (SOD) (Fig. 1) (32, 92). Events that impede the flow of electrons through the ETC will enhance the rate of superoxide production by increasing the half-life of reduced components within the ETC.

There are two mechanisms by which obesity may induce an increase in mitochondrial superoxide anion and \( \text{H}_2\text{O}_2 \) production. The first invokes increased oxidation of energetic substrates and, thus, enhanced production of reducing equivalents that enter the ETC. In the absence of increased energy demand, reduced components of the ETC have a greater probability of catalyzing the incomplete reduction of \( \text{O}_2 \) to form superoxide anion. However, the generation and utilization of reducing equivalents in the form of NADH and FADH\(_2\) involve a series of highly regulated processes to maintain redox homeostasis. Evidence to indicate that this delicate balance is disrupted in response to obesity is lacking. The second mechanism takes into account events that induce a metabolic shift toward increased reliance on fat relative to glucose for energy production. Such a shift occurs in heart and skeletal muscle with high dietary fat and obesity due to increases in circulating free fatty acids (57, 65). Independent of the number of calories ingested or energy demand, mitochondrial \( \text{H}_2\text{O}_2 \) production is inherently greater when fatty acids relative to the glycolytic metabolite pyruvate are oxidized (5, 75, 83). Oxidation of both fatty acids and pyruvate within the mitochondria results in the production of acetyl-CoA for use in the reduction of NAD\(^+\)
Fig. 1. Enhanced mitochondrial H$_2$O$_2$ production due to increased utilization of fatty acids. Within the mitochondria, fatty acids and pyruvate are oxidized to generate acetyl-CoA for use by the Krebs cycle. Energetic substrate oxidation produces reducing equivalents in the form of NADH and FADH$_2$ that donate electrons to the electron transport chain (ETC). In addition, fatty acid oxidation ($\beta$-oxidation) results in transfer of electrons to the ubiquinone pool (Q) via the electron transport flavoprotein (ETF). The entry of electrons downstream of complex I has the potential to create back pressure on the flow of electrons through complex I. This change in electron flow enhances the probability of single electron emission from the ETC and incomplete reduction of oxygen to superoxide anion, which is rapidly converted to H$_2$O$_2$ by superoxide dismutase (SOD). Thus increased oxidation of fatty acids in response to high dietary fat and obesity can lead to increased mitochondrial H$_2$O$_2$ production.

and FAD$^+$ within the Krebs cycle. However, in contrast to pyruvate oxidation, the first step in fatty acid oxidation ($\beta$-oxidation) involves the transfer of electrons from FADH$_2$ formed by acyl-CoA dehydrogenases to the electron transport flavoprotein. Electrons are then donated from the electron transport flavoprotein directly to the ubiquinone pool, downstream of complex I, the entry point for electrons from NADH into the ETC. In this scenario, complex I would be in a more reduced state, increasing the likelihood for release of single electrons that catalyze the incomplete reduction of O$_2$ to superoxide anion, which is rapidly converted to H$_2$O$_2$ (Fig. 1) (32). In fact, we have shown that isolated cardiac mitochondria exhibit significantly higher rates of H$_2$O$_2$ generation when respiring on palmitoyl carnitine relative to pyruvate (75). Furthermore, this phenomenon has been observed in skeletal muscle mitochondria and permeabilized fibers (5, 83). Increased reliance on $\beta$-oxidation also leads to accumulation of various lipophilic fatty acid-derived intermediates. In vitro studies reveal that certain fatty acid derivatives can interact with the mitochondrial inner membrane, disrupt electron transport, and stimulate mitochondrial free radical production (17, 36, 81, 83, 103). Thus, differences in the relative utilization of carbohydrate versus fat can generate unique redox signatures within the mitochondria.

The concept of unique redox signature becomes important during physiological increases in the concentration of circulating triglycerides and free fatty acids during high-fat feeding or starvation. Under these conditions, both the heart and skeletal muscle exhibit a metabolic shift toward increased reliance on fat rather than glucose for energy production (58, 71). Thus one would predict that high dietary fat increases mitochondrial production of free radicals in both cardiac and mixed skeletal muscle. Experimentally, increased mitochondrial H$_2$O$_2$ production has been shown in skeletal muscle (4), adipose tissue (19), and kidney (77) of mice fed a high-fat diet. Suppression of $\beta$-oxidation (52) or selective scavenging of mitochondrial H$_2$O$_2$ (4, 14) preserves skeletal muscle insulin sensitivity in models of diet-induced obesity. In the heart, suppression of $\beta$-oxidation has a similar protective effect against high-fat diet-induced insulin resistance (93, 105). Therefore, the development of insulin resistance as a molecular consequence of enhanced mitochondrial H$_2$O$_2$ production in response to increased reliance on fatty acids for energy production appears to be a general characteristic of multiple tissues including the heart. Studies on the effect of high-fat diet on insulin sensitivity in skeletal muscle often employ tissue that contains both fast- and slow-twitch muscle fibers. However, evidence indicates that oxidative slow-twitch skeletal muscle, which is metabolically similar to the heart, is more susceptible than glycolytic fast-twitch skeletal muscle to diet-induced insulin resistance (18, 46, 87, 106).

Mitochondrial Fatty Acid Oxidation and Insulin Resistance

Long-chain fatty-acid derivatives and triacylglycerol that accumulate in skeletal muscle and heart due to impaired mitochondrial $\beta$-oxidation are widely believed to be the causative agents of the insulin resistance seen with obesity (64, 76). These lipids are used to synthesize the signaling intermediates diacylglycerol and ceramide that can activate stress responsive serine kinases and, thus, negatively regulate insulin signaling (45, 102). Based on this scenario, the appropriate therapeutic strategy for preserving insulin sensitivity is to increase mitochondrial fatty acid oxidation to prevent the accumulation of intramuscular triacylglycerol (16, 97). However, recent evidence indicates that $\beta$-oxidation rapidly increases in response to high dietary fat (41, 58, 88, 100) and that inhibition rather than activation of fat consumption is cardioprotective (52, 57, 58, 65, 93). Long-chain acyl-CoA molecules are converted to alycamines by carnitine palmitoyltransferase 1 before mitochondrial import. Malonyl-CoA decarboxylase (MCD) degrades malonyl-CoA, which is a potent inhibitor of carnitine palmitoyltransferase 1. Mice that lack MCD have a reduced ability to import and oxidize long-chain fatty acids within the
mitochondria. Indeed, the increase in mitochondrial fatty-acid oxidation normally observed in the heart and skeletal muscle of mice fed a high-fat diet was attenuated in MCD-deficient mice (52, 93). Reduced fatty-acid oxidation as a result of MCD deficiency actually improved cardiac insulin sensitivity in mice fed a high-fat diet despite significantly elevated myocardial triacylglycerol content (93, 105). Furthermore, transgenic overexpression of diacylglycerol acyltransferase-1 has been shown to enhance intramuscular triglyceride content while also improving skeletal muscle insulin resistance in high fat-fed mice (55). Together, these data indicate that it is not accumulation of triacylglycerol in cardiac and skeletal muscle that drives high-fat diet-induced insulin resistance but, rather, the subsequent increased utilization of fatty acids by the mitochondria for energy production.

In a complementary set of experiments, mice that lack peroxisome proliferator-activated receptor-α (PPAR-α), a transcription factor that drives expression of genes responsible for transport and oxidation of fatty acids (20), are resistant to high-fat diet-induced insulin resistance (29, 40). Whole body PPAR-α knockout mice retained normal insulin secretion from pancreatic islets ex vivo but did not show the high-fat diet-induced hyperinsulinemia seen in wild-type controls (52). PPAR-α knockout mice gain more weight on a high-fat diet than wild-type controls, have a reduced ability to oxidize palmitate, and show high tissue triglyceride levels. Glucose tolerance and whole body insulin-stimulated glucose uptake, however, are largely preserved in response to high dietary fat, consistent with a sparing of insulin function (29, 40). Heart and skeletal muscle-specific PPAR-α overexpression increased the expression of a number of fatty acid metabolism genes and reduced glucose uptake and utilization, inducing a metabolic phenotype similar to that seen in diabetes (29, 30). These data, along with the data from the MCD knockout mice, support mitochondrial β-oxidation as a source of the signal(s) that promote high-fat diet-induced insulin resistance in cardiac as well as skeletal muscle.

Mitochondrial-Derived H$_2$O$_2$ as a Mediator of Insulin Resistance

Evidence indicates that mitochondrial-derived H$_2$O$_2$ is a likely signal for modulating insulin sensitivity. As previously discussed, one would expect mitochondrial H$_2$O$_2$ production to increase in response to high-circulating free fatty acids and increased reliance on β-oxidation for energy production. Thus the level of H$_2$O$_2$ would be reflective of increased utilization of fatty acids by mitochondria and represents a potential signal for diminishing insulin signaling. This contention is supported by results from experiments using transgenic mice in which H$_2$O$_2$ consuming antioxidant enzymes were specifically overexpressed in the mitochondria.

Superoxide anion generated by the ETC is rapidly converted to H$_2$O$_2$ by SOD. Intracellular H$_2$O$_2$ is consumed either by catalase, peroxiredoxin (PRDX), or glutathione peroxidase (GPx). Overexpression of mitochondrial SOD has been shown to improve insulin-dependent skeletal muscle glucose uptake and whole body insulin sensitivity in high fat-fed rodents, suggesting a role for superoxide anion in the negative regulation of insulin sensitivity (10, 44). Similarly, mice that overexpress H$_2$O$_2$ consuming enzymes within the mitochondria also resist high-fat diet-induced insulin resistance. Indeed, transgenic overexpression of mitochondrial PRDX3 has been shown to reduce H$_2$O$_2$ production in skeletal muscle mitochondria, reduce blood glucose levels, and protect against high-fat diet-induced insulin resistance (14). Additional support for an inhibitory role of mitochondrial H$_2$O$_2$ production in regulating insulin sensitivity is provided by work with transgenic mice that overexpress human catalase engineered to localize in the mitochondria (mCat). Relative to wild-type counterparts, these mCat mice exhibit lower rates of mitochondrial H$_2$O$_2$ production and, in response to high dietary fat, improved insulin sensitivity in skeletal muscle (4). Although superoxide anion itself may have an independent role in regulating insulin sensitivity, the combination of the specificity of catalase for H$_2$O$_2$ and targeting of the enzyme to the mitochondria strongly suggests that elevated H$_2$O$_2$ production, as a result of increased reliance of mitochondria on fatty acid oxidation, diminishes insulin sensitivity. Proof requires elucidation of mechanisms by which mitochondrial-derived H$_2$O$_2$ inhibits insulin signaling.

Redox Regulation of Insulin Signaling

H$_2$O$_2$ can exert stimulatory or inhibitory effects on insulin signaling, depending on the concentration of H$_2$O$_2$ and/or the site of production relative to various components of insulin signaling pathway(s) (Fig. 2). Insulin binding induces tyrosine phosphorylation of the insulin receptor substrate (IRS), leading to Akt phosphorylation that culminates in phosphorylation of the Rab GTPase-activating protein AS160 and translocation of the glucose transporter Glut4 to the plasma membrane (9). In skeletal muscle and adipose tissue, it has been shown that insulin receptor binding also increases the activity of Nox present in the plasma membrane, leading to increased H$_2$O$_2$ concentration proximal to the receptor (24, 61). Nox-derived H$_2$O$_2$ has been shown to inhibit as well as enhance insulin sensitivity. Indeed, cultured cardiomyocytes treated with high glucose display impaired insulin signaling that is rescued by knockdown of Nox2 (7). In contrast, Nox4 knockout mice exhibit greater insulin resistance than wild-type mice when fed a high-fat diet (54). Additional support for a role of Nox in the enhancement of insulin sensitivity comes from GPx1 knockout mice that resist high-fat diet-induced whole body insulin resistance (56). Insulin-stimulated phosphorylation of Akt is greater in the GPx1$^{-/}$ cells and is suppressed by treatment with the Nox inhibitor diphenylene iodonium chloride (56). Furthermore, insulin-stimulated phosphatase and tension homolog (PTEN) oxidation is also elevated in GPx1$^{-/}$ cells and is suppressed in Nox inhibition. The protein tyrosine phosphatases PTP-1B and PTEN, known to inhibit insulin signaling (23, 99), are transiently oxidized and inactivated by H$_2$O$_2$ (91). The data from GPx1$^{-/}$ mice support the ability of H$_2$O$_2$, produced by Nox, to stimulate the insulin signaling cascade by inhibiting protein tyrosine phosphatases.

The mechanism(s) by which mitochondrial H$_2$O$_2$ generation impairs insulin signaling are not fully characterized, but several proteins have been identified as potential effectors (Fig. 2). One family of candidate proteins is the stress-sensitive Ser/Thr kinases. c-Jun NH$_2$-terminal kinase 1 (JNK1) in particular has emerged as an important component in the development of insulin resistance. JNK1 is capable of inhibiting IRS activity via phosphorylation of Ser307, a post-translational modification
that prevents interaction of IRS with the insulin receptor (2, 3). Indeed, high dietary fat induced increases in body mass and blood glucose levels, and loss of insulin sensitivity are diminished in JNK1 knockout mice relative to wild-type control animals (43). Although a direct link between mitochondrial H$_2$O$_2$ production and JNK1 activation remains to be demonstrated, an inverse relationship between PTP-1B activity and JNK1 activation has been shown in H4IIEC hepatocytes treated with increasing concentrations of H$_2$O$_2$ (50). PTP-1B activity was inhibited and insulin stimulated IRS and AKT phosphorylation were reduced. These data illustrate the relationship between subtle changes in H$_2$O$_2$ concentration and fine modulation of insulin sensitivity. Thus, given that suppression of β-oxidation (52, 93) or overexpression of mitochondrial antioxidant enzymes that consume H$_2$O$_2$ (4, 14) diminish high dietary fat-induced loss of insulin sensitivity, increased H$_2$O$_2$ production by the mitochondria may be a major contributor to insulin resistance associated with obesity. In this scenario, mitochondria serve as a refined sensor of changes in substrate availability, adjusting metabolism to optimize use and clearance of available substrate(s).

Mitochondria as a Sensor of Nutritional Status and Regulator of Metabolism

Evolutionary pressure on human and other mammals has selected for efficient use and partitioning of energetic substrates. Mitochondria are uniquely positioned to sense changes in dietary composition and mount an appropriate metabolic response. Muscle tissue, for example, can regulate a shift in substrate utilization based on availability and energy demand. When circulating levels of fatty acids are high, such as upon consumption of high dietary fat and during chronic obesity, mitochondria exhibit enhanced rates of fatty acid oxidation (58). The glucose-fatty acid cycle, also termed the Randle Cycle (Fig. 3), describes the allosteric regulation of energetic substrate selection in which products of fatty acid oxidation inhibit glucose utilization for energy production (70). Increased production of acetyl-CoA from fatty acid oxidation activates pyruvate dehydrogenase kinase, inhibiting the pyruvate dehydrogenase-mediated generation of acetyl-CoA from pyruvate (49, 70). However, allosteric regulation of substrate use within the mitochondria does not prevent continued uptake of glucose. Inappropriate intracellular accumulation of glucose induces damage to the microvasculature, with functional consequences for cardiac and skeletal muscle (37). It would therefore be beneficial to rapidly and reversibly inhibit insulin signaling and appropriately adjust energy metabolism within cells. We would argue that a subtle increase in mitochondrial H$_2$O$_2$ generation resulting from the increased fatty acid oxidation is able to slow the uptake of glucose by downregulating insulin sensitivity. While the inhibitory effect of H$_2$O$_2$ on insulin sensitivity is traditionally considered deleterious, H$_2$O$_2$ is also an important signaling molecule (73, 94). Modulation of mitochondrial H$_2$O$_2$ production by oxidation of different energetic substrates may serve as both a sensor of nutritional status and an important intracellular signal to appropriately adjust metabolism. Indeed, mitochondrial free radical production has also been shown to enhance uncoupling protein (UCP) activity (13, 22). Increased UCP3 expression in L6 muscle cells enhances fatty acid oxidation (60), which, in the context of high dietary fat, would help to ensure efficient fatty acid utilization to prevent the buildup of toxic lipid intermediates. As such, redox regulation of both insulin sensitivity and UCP activity may complement the Randle Cycle in modulating substrate utilization. Thus a short-term metabolic shift toward increased fatty acid oxidation may represent a regulated and beneficial response that, with chronic obesity, manifests as insulin resistance, diabetes, and cardiovascular disease.

The Role of Antioxidants in H$_2$O$_2$-Mediated Insulin Signaling

If inhibition of insulin signaling by mitochondrial-derived H$_2$O$_2$ is beneficial, then endogenous antioxidants would represent important components of the process. The level of various antioxidants must be delicately balanced to provide...
appropriate levels and molecular species for redox regulation while preventing oxidative damage (Fig. 4). This is particularly important when one considers that antioxidants exhibit distinct molecular properties, specificity for oxidant species, and locations within the cell. For enzymes that consume H$_2$O$_2$, the binding affinity of catalase is orders of magnitude lower than that of PRDX and GPx (74). The low-binding affinity of catalase makes it ideal for consuming potentially toxic levels of H$_2$O$_2$ while not perturbing lower levels of H$_2$O$_2$ required for signaling. Antioxidant enzymes that have affinities for H$_2$O$_2$ in the physiological range can then participate in the fine regulation of insulin signaling. As previously discussed, evidence indicates that H$_2$O$_2$ production at the plasma membrane (via Nox) and mitochondria is capable of enhancing and diminishing insulin sensitivity, respectively. These findings illustrate the importance of location in both H$_2$O$_2$ production and antioxidant expression. As exemplified in the PRDX3 and mCat transgenic mice, mitochondrial localized antioxidant enzymes can prevent diet-induced loss in insulin sensitivity (4, 14). In contrast, mice lacking GPx1, a ubiquitously expressed isoform of GPx, exhibit increased insulin sensitivity in response to high-fat diet relative to wild-type control animals (56). We have demonstrated that endogenous catalase concentration and activity are significantly elevated in the hearts of mice maintained on high-fat diet for one day (75). Furthermore, catalase was found to be present and significantly elevated within cardiac mitochondria in high fat-fed mice. While the observed increase in endogenous catalase concentration and activity are significantly elevated in the hearts of mice maintained on high-fat diet for one day (75). Furthermore, catalase was found to be present and significantly elevated within cardiac mitochondria in high fat-fed mice. While the observed increase in endogenous catalase concentration and activity are significantly elevated in the hearts of mice maintained on high-fat diet for one day (75). Furthermore, catalase was found to be present and significantly elevated within cardiac mitochondria in high fat-fed mice. While the observed increase in endogenous catalase concentration and activity are significantly elevated in the hearts of mice maintained on high-fat diet for one day (75). Furthermore, catalase was found to be present and significantly elevated within cardiac mitochondria in high fat-fed mice. While the observed increase in endogenous catalase concentration and activity are significantly elevated in the hearts of mice maintained on high-fat diet for one day (75). Furthermore, catalase was found to be present and significantly elevated within cardiac mitochondria in high fat-fed mice. While the observed increase in endogenous catalase concentration and activity are significantly elevated in the hearts of mice maintained on high-fat diet for one day (75). Furthermore, catalase was found to be present and significantly elevated within cardiac mitochondria in high fat-fed mice.
while allowing PRDX and GPx enzymes to maintain physiological concentrations of H2O2 used for signaling.

An essential consideration is that overlapping regulatory pathways coordinate insulin signaling, H2O2 production, and expression of certain antioxidant enzymes. Specifically, forkhead box O (FoxO) transcription factors drive the expression of catalase (89) and PRDX3 (15) in cultured cardiac cells. Foxo activity is suppressed by insulin signaling in cardiomyocytes (89). It is therefore notable that treatment of cells with H2O2 induces the translocation of forkhead box transcription factor Foxo4 to the nucleus in a JNK1/2-dependent manner (25). These results suggest that H2O2-mediated JNK activation would not only diminish insulin signaling but, in so doing, activate Foxo-dependent transcription of antioxidant enzymes. These synchronized events could provide for appropriate buffering of H2O2 to ensure redox regulation of insulin signaling while preventing oxidative damage. Furthermore, this buffering system would also allow for rapid restoration of insulin sensitivity when circulating lipid levels decline and lipid-dependent mitochondrial H2O2 production is attenuated.

Summary and Future Perspective

High-caloric diets in the absence of increased energy demand induce a rapid metabolic shift toward increased reliance on fatty acid oxidation, particularly in the heart and skeletal muscle (58, 65). One might therefore predict that diminished pyruvate utilization and enhanced β-oxidation by the mitochondria would precede loss of insulin sensitivity. This prediction is based on rapid allosteric regulation, as described by the Randle Cycle (49, 70), and activation of a transcriptional program that favors β-oxidation over pyruvate utilization in response to increased circulating triglycerides and free fatty acids (47, 85, 86, 101, 104). Thus, it is critical to determine how the metabolic shift toward increased fatty acid oxidation drives the progression to insulin resistance and cardiovascular disease. This information is necessary to define effective dietary interventions, clearly articulate health incentives, and discern novel diagnostic and therapeutic targets for reduction or prevention of heart disease. To capitalize on our knowledge of what can be considered a metabolic disease, future studies must address several lines of investigation.

1) Mitochondrial H2O2 production is inherently greater during oxidation of fatty acids relative to pyruvate (5, 75, 83). In model systems, inhibition of mitochondrial H2O2 production (4, 14) or fatty acid utilization (52, 93) reduces diet-induced loss in insulin sensitivity. It is therefore important to establish molecular mechanisms by which mitochondrial H2O2 production exerts distinct effects on specific components of insulin signaling cascades in physiologically relevant models and in humans. JNK1 represents an attractive candidate to directly link mitochondrial H2O2 production to loss in insulin sensitivity (50). Furthermore, given that prooxidants are both signaling and reperfusion (58). Nevertheless, organisms require the capacity to rapidly shift utilization of energetic substrates based on fuel availability and demand, as illustrated by the Randle Cycle (49, 70). Indeed, enhanced mitochondrial utilization of fatty acids inhibits glucose and pyruvate oxidation through regulated allosteric and post-translational mechanisms (49, 70). This shift in energetic substrate utilization occurs rapidly, as illustrated by acute effects of starvation on mitochondrial energy metabolism (47, 85) and circadian fluctuations in the levels of specific components of fatty acid and glucose metabolism (86, 101). Future studies must identify the time frame in which changes in energetic substrate preference exert effects on insulin signaling and the reversibility of distinct alterations. An important experimental consideration is that the divergent nutritional statuses of fasting and high dietary fat both elicit increases in circulating triglycerides and, as such, induce similar alterations in certain measures of metabolism (58, 71). Thus fasting experimental animals before assessing metabolic effects of defined dietary regimens may obscure underlying causes or the magnitude and progression of alterations in metabolism (6). Finally, nearly 30% of the obese population is metabolically healthy and does not develop obesity-associated metabolic diseases (11, 28, 72, 84). Identification of molecular mechanisms responsible for differences in healthy and unhealthy obese individuals will enable refinement of therapeutic targets and the effectiveness of interventional strategies.

2) While allowing the exploitation of the antioxidant system in the regulation of insulin sensitivity.

3) Metabolic flexibility, defined as the ability to preferentially use fatty acids or pyruvate for energy production, would be considered beneficial in the context of starvation, where both the supply of circulating fatty acids and the need to spare glucose is high. It could be argued that increased reliance on fatty acids and inhibition of insulin signaling is also beneficial with high-fat diet, enabling preferential consumption of fatty acids while limiting the potentially damaging effects of glucose accumulation (i.e., glucose toxicity). Alternatively, the response to high circulating fat, while appropriate during periods of starvation, may be conserved but inappropriate in the context of high dietary fat. Defining the consequences of preventing diet-induced alterations in metabolism on tissue and cellular function will allow assessment of short- and long-term advantages or disadvantages. It will also be critical to deter-
mine nutritional scenarios (i.e., composition, duration, fluctuations) in which a potentially beneficial response becomes deleterious resulting in metabolic inflexibility where the heart can no longer appropriately switch between energetic substrates.

Insulin signaling exerts tissue-specific effects on a multitude of intracellular processes. This complexity necessitates that an intricate network of pathways and effectors appropriately regulate insulin signaling to reflect energy demand and substrate availability. Within this network, the term insulin resistance invokes a presumably unwanted effect of impaired function. Based on the data presented here, however, it is reasonable to argue a more favorable view of insulin resistance as a normal response to dietary conditions where glucose metabolism must be downregulated to promote fatty acid metabolism and prevent glucose toxicity or, in the case of starvation, spare glucose for glycolytic tissue. In the context of high dietary fat, increased reliance on and utilization of fatty acids within the mitochondria appear to serve as the stimuli for induction of insulin resistance. While high-fat diet-induced obesity also increases intramuscular triglyceride accumulation, evidence suggests that this phenomena does not contribute to the regulation of insulin sensitivity. It is perhaps the chronic consumption of excess calories, particularly in the form of fatty acids, coupled with the ensuing unnatural state of obesity that turns normal regulation of insulin sensitivity into an unwanted metabolic abnormality. Elevated circulating lipid levels inherent to insulin resistance increases intracellular triglyceride accumulation, evidence which indicates this phenomena does not contribute to the regulation of insulin sensitivity. It is perhaps the chronic consumption of excess calories, particularly in the form of fatty acids, coupled with the ensuing unnatural state of obesity that turns normal regulation of insulin sensitivity into an unwanted metabolic abnormality. Elevated circulating lipid levels inherent to obesity promote long-term insulin resistance, elevated blood glucose levels, and downstream complications such as type 2 diabetes and cardiovascular disease.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES


