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COMMENTARY

An oxidative stress paradox: time for a conceptual change?

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Abstract Oxidative stress has long been considered a key driving factor of many obesity-related health problems. However, recent work by Merry, Tran et al (*Diabetologia* DOI 10.1007/s00125-016-4084-3) challenges this idea with an interesting study using a hepatocyte-specific *Gpx1*-knockout (HGKO) mouse. GPX1 is an important detoxification enzyme that converts H_2O_2 to water. The authors found that high-fat diet-fed HGKO mice were more insulin sensitive than wildtype controls, despite elevated hepatic levels of H_2O_2 and evidence of increased systemic oxidative stress. When challenged with a non-alcoholic steatohepatitis (NASH)-inducing diet, HGKO mice were also protected, displaying reduced levels of inflammation and fibrosis with similar levels of steatosis compared with controls. These findings call into question the role of reactive oxygen species in NASH pathogenesis and highlight a potential paradox whereby increased H_2O_2 may be beneficial in some contexts.

Keywords Glutathione peroxidase · Insulin resistance · Liver · Non-alcoholic fatty liver disease · Non-alcoholic steatohepatitis · Oxidative stress · Reactive oxygen species

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Abbreviations

CDAA	Choline-deficient amino-acid-defined
GPX	Glutathione peroxidise
HGKO	Hepatocyte-specific *Gpx1*-knockout
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
ROS	Reactive oxygen species

Oxidative stress has long been considered an important factor driving obesity-related insulin resistance and its pathophysiological consequences. In this issue of *Diabetologia*, a study from Merry, Tran, et al [1] refutes this over-simplistic idea and highlights the beneficial effects of reduced antioxidant capacity through the hepatic loss of glutathione peroxidase (GPX)1. The authors show that despite elevated H_2O_2 levels, hepatocyte-specific *Gpx1*-knockout (HGKO) mice were more insulin sensitive and had better whole body glucose metabolism. These effects on hepatic insulin signalling were also present when mice were challenged with a high-fat diet. Though insulin sensitivity was not specifically assessed, HGKO mice on the choline-deficient amino-acid-defined (CDAA) diet (which induces a non-alcoholic steatohepatitis [NASH]-like pathology), displayed reduced severity of inflammation and fibrosis, without influencing steatosis. These findings run contrary to the prevailing dogma that H_2O_2 overproduction is an obligate precursor to insulin resistance, NASH and other pathologies.

Reactive oxygen species (ROS) are produced as part of normal cellular function. Superoxide anion ($O_2^{\bullet -}$), the most potent ROS compound, has several cellular sources. It is a natural byproduct of the electron transport chain in mitochondria, as part of glucose or fatty acid oxidation, and is also produced by membrane-associated oxidases, such as NADPH oxidase, as well as by cytosolic endoplasmic

reticulum and peroxisomal oxidases. Normally, superoxide is converted to the less reactive H_2O_2 by the superoxide dismutase (SOD) enzymes. H_2O_2 can then be converted to water by a number of enzymes, including GPXs. These enzymes use glutathione to convert H_2O_2 to water, thereby producing oxidised glutathione (GSSG). H_2O_2 is also generated as part of the normal signal transduction of many hormones, notably insulin itself [2], as well as cytokines such as IL-6, TNFα and others [3]. It is currently unknown whether there are specific mechanisms for handling metabolic vs signalling-induced ROS, or how these pathways might interact, but it can be postulated that the origin of ROS may differentially modulate physiological vs pathological pathways.

In diseased states, the combination of continuous oxidation of abundant energy substrates and dysregulation of many signalling pathways is thought to overwhelm the antioxidant system, resulting in oxidative stress [4]. Excessive ROS can drive cellular dysfunction through direct (i.e. signalling) and indirect (e.g. protein, lipid or DNA modification) mechanisms. Evidence in support of this hypothesis comes from numerous association studies in obese humans, demonstrating positive correlations between systemic markers of oxidative stress and BMI [5]. Moreover, ROS production has been demonstrated to play a key role in mediating TNFα and glucocorticoid-induced insulin resistance in adipocytes [6]. Evidence of oxidative stress is also present in more advanced stages of metabolic disease, for example in NASH-affected livers [7] and in the vascular wall in atherosclerosis [8]. It is not surprising, then, that the theory of oxidative stress-induced metabolic deterioration has gained wide attention.

One central question related to the H_2O_2-metabolising GPXs is whether their function is more important in normal physiological states or in the protection against oxidative stress in disease. GPX1 and GPX4 are the major isoforms in liver, with GPX1 accounting for nearly all cytosolic and mitochondrial GPX activity [9]. While *Gpx4*-knockout mice are not viable, global *Gpx1*-knockout mice are viable and are less susceptible to high-fat diet-induced insulin resistance [10]. This effect was attributed to enhanced insulin-stimulated glucose uptake in the muscle of knockout animals. However, the nature of the global knockout makes it difficult to determine the various organ level effects, and how localised oxidative stress may affect systemic metabolism.

To address this question, the authors generated an HGKO mouse and assessed variables of hepatic and systemic glucose metabolism. Under normal physiological conditions, HGKO mice showed no evidence of systemic or hepatic oxidative stress and were moderately more insulin sensitive. In clamp studies, HGKO mice had a higher glucose infusion rate and a trend towards higher disappearance rate, together suggesting enhanced peripheral insulin sensitivity and glucose uptake. In conjunction with reduced hepatic gluconeogenic gene expression, this led to reduced fasting glucose and insulin levels in the HGKO mice, compared with controls. The authors went

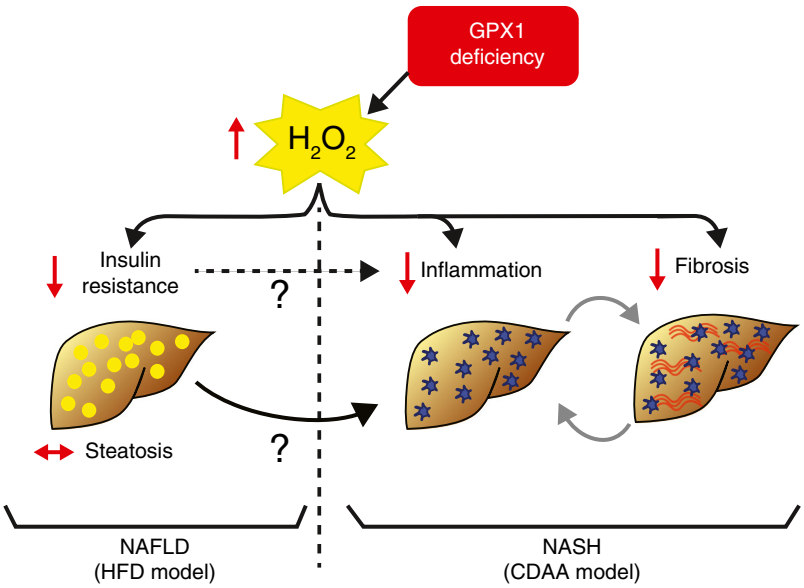

Fig. 1 Proposed actions of oxidative stress in NAFLD. Oxidative stress is thought to be central to the natural history of NAFLD. Enhanced oxidative stress can influence insulin sensitivity, which can lead to steatosis. In the insulin resistant state, enhanced sensitivity to the development of NASH and fibrogenesis is being observed though the mechanisms remain poorly characterised. HGKO mice, devoid of hepatic GPX1, show enhanced H_2O_2 production and accumulation, yet remain more insulin sensitive and are protected from inflammation and fibrosis. Thus, reduced antioxidant capacity via hepatic GPX1 loss may be beneficial in the context of insulin resistance and liver disease. HFD, high-fat diet. Red arrows indicate physiological changes associated with hepatic GPX1 deficiency. The dashed arrow indicates the suggestion that insulin resistance may enhance progression from steatosis to NASH. Black arrows indicate direct influence and disease progression. The dashed line separates the two experimental models used to probe NAFLD and NASH

on to demonstrate that insulin-induced insulin receptor and Akt phosphorylation were increased and that these increases were potentiated for a longer period of time in livers of HGKO mice, compared with controls. When challenged with a high-fat diet, HGKO showed elevated levels of systemic oxidative stress as well as increased hepatic H_2O_2. Despite this, high-fat diet-fed HGKO mice displayed many of these same features of enhanced insulin sensitivity when compared with high-fat-fed controls. Overall, these findings suggest that hepatic GPX1 is more important in the modulation of insulin signalling, rather than in the response to metabolic stress.

Finally, the authors turned their attention to one of the central questions in non-alcoholic fatty liver disease (NAFLD): how does modulation of oxidative stress modify its natural history and progression (Fig. 1)? Patients with diabetes are much more likely to have steatosis and NASH than the general population, yet a clear reason for this link remains undetermined [11]. Here, the authors challenged mice with a CDAA diet, which induces NASH pathology in the context of normal weight gain (not obesity) [12]. Despite relatively small numbers of mice, they surprisingly found that HGKO mice had less lobular inflammation and circulating levels of the inflammatory cytokines, TNFα, IL-6 and IFN-γ, with similar levels of steatosis. With respect to fibrogenesis, where oxidative stress is thought to be a key driving factor, HGKO were also apparently protected. Hepatic mRNA levels of fibrogenic genes and histology scoring were both moderately improved in HGKO compared with control mice. These findings suggest that altered H_2O_2 signalling pathways in HGKO mice delay the onset of fibrosis in NASH. Importantly, the HGKO mouse is yet another model showing that NASH and fibrosis progression can occur separate from changes in steatosis, as also previously shown using peroxisome proliferator-activated receptor (PPAR)α signalling models [13]. It will be important to study the relative contribution of insulin vs other signalling pathways to the effects of GPX1-induced H_2O_2 on NASH.

How can we begin to reconcile these apparently paradoxical findings? Data from both global and liver specific knockout models suggest that GPX1 acts primarily to attenuate insulin signalling. Indeed, the authors show that the absence of GPX1 leads to enhanced oxidation (and thus inactivation) of protein phosphatases that would otherwise suppress the insulin (and likely other) signalling cascades. There is precedent for such a mechanism. Studies performed in adipose tissue have also demonstrated beneficial (and necessary) effects of enhanced H_2O_2 production [14]. It is also important to consider that compared with $O_2•^-$ and $HO•^-$, H_2O_2 is much less oxidative. The present study suggests that the more active ROS may be more relevant in disease-associated oxidative stress.

What is next in the debate on the role of oxidative stress in driving metabolic disease? In the present study, the authors have explored relatively short-term interventions. It will be interesting to evaluate the long-term effects of GPX1-deficiency on ageing processes, where prolonged exposure to H_2O_2 may become detrimental. Indeed, diseases like type 2 diabetes, atherosclerosis and NASH evolve over the course of years or decades in humans. Additionally, the role of oxidative stress in driving NASH pathology (especially fibrosis) is much less proven in the CDAA model. By contrast, the strongly fibrogenic CCl_4 model drives fibrogenesis as a result of ROS production during its metabolism by cytochrome P450 2E1 (CYP2E1). Future studies are thus needed to determine whether the protection of HGKO mice from advanced NASH pathology indeed arises from their enhanced insulin sensitivity; this would be an important advance in our understanding of the connection between type 2 diabetes and NAFLD. In the meantime, this elegant study again warns against over-simplistic models of the role of oxidative stress in metabolic disease and provides a potential explanation for the failure of non-specific antioxidant strategies against metabolic diseases.

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