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Original Contribution

# Knockout of SOD1 alters murine hepatic glycolysis, gluconeogenesis, and lipogenesis

Li Wang [a,b], Zongyong Jiang [b], Xin Gen Lei [a,*]

[a] Department of Animal Science, Cornell University, Ithaca, NY 14853, USA
[b] Key Laboratory of Animal Nutrition and Feed Science (South China) of the Ministry of Agriculture, Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangzhou, Guangdong 510640, China

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ABSTRACT

We previously observed a stronger effect of knockout of Cu,Zn-superoxide dismutase (SOD1) than that of Se-dependent glutathione peroxidase 1 (GPX1) on murine body weight and glucose homeostasis. Two experiments were conducted to determine how hepatic lipid profiles and key metabolic regulators were correlated with this difference. SOD1$^{-/-}$ and GPX1$^{-/-}$ mice and their respective wild-type (WT) littermates ($n=6$ or 7/group, male) were fed a Se-adequate Torula yeast–sucrose diet and killed at 6 months of age to collect liver samples. In Experiment 1, fasted SOD1$^{-/-}$ mice displayed pyruvate intolerance and a 61% decrease ($P<0.05$) in liver glycogen compared with their WT littermates. The former had lower ($P<0.05$) activities of phosphoenolpyruvate carboxykinase, total protein phosphatase, and protein phosphatase 2A, but a higher ($P<0.05$) activity of glucokinase in the liver than the latter. In contrast, hepatic concentrations of total cholesterol, triglycerides, and nonesterified fatty acids were increased by 11 to 100% ($P<0.05$) in the SOD1$^{-/-}$ mice. Meanwhile, these mice had elevated ($P<0.05$) hepatic protein levels of sterol-regulatory element binding proteins 1 and 2, p53, total and phosphorylated AMP-activated protein kinase α1 protein, protein tyrosine phosphatase 1B, and protein phosphatase 2B. In Experiment 2, GPX1$^{-/-}$ mice and their WT littermates were compared, but showed no difference in any of the measures. In conclusion, knockout of SOD1, but not GPX1, led to a decreased liver glycogen storage synchronized with pyruvate intolerance and elevated hepatic lipid profiles in adult mice. This striking comparison was possibly due to unique impacts of these two knockouts on intracellular tone of $H_2O_2$ and key regulators of liver gluconeogenesis, glycolysis, and lipogenesis.

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## Introduction

Metabolic syndrome (MS)[1] is a complex disorder with multiple interrelated disturbances of glucose and lipid homeostasis [1]. The prevalence of MS is increasing to epidemic magnitude, afflicting not only the United States and other urbanized countries but also developing nations. Major risk factors of MS include abdominal obesity [2], atherogenic dyslipidemia [3], hepatic steatosis [4], and dysregulation of glucose metabolism [5]. Many studies show that MS and its associated diseases are often involved in oxidative stress, a condition featured as accelerated production and/or attenuated removal of reactive oxygen species (ROS) [5,6]. Despite their essential role in multiple physiological systems, ROS, at excessive levels, lead to cellular dysfunction in excess [7].

Cu,Zn-superoxide dismutase (SOD1) and Se-dependent cellular glutathione peroxidase 1 (GPX1) are widely considered to be two primary intracellular antioxidant enzymes in mammals. SOD1 can convert superoxide anion to hydrogen peroxide [8], which is subsequently reduced to water with the catalysis of GPX1 [9]. Because of this coupled reaction, SOD1 and GPX1 are thought to be cooperative in protecting cells against oxidative stress [10–12]. Our laboratory has used SOD1$^{-/-}$ and GPX1$^{-/-}$ mice to study the metabolic roles of these two enzymes in hepatic oxidative stress or drug toxicity [13], bone metabolism [14], and pancreatic physiology [15]. These studies reveal a greater impact from knockout of SOD1 than from GPX1 on adult body weight, femoral mechanical characteristics, islet function, pancreas integrity, and

*Abbreviations:* AMPK, AMP-activated protein kinase; GK, glucokinase; GPX1, Se-glutathione peroxidase 1; JNK2, c-Jun $NH_2$-terminal kinase; MAPK, mitogen-activated protein kinase; MS, metabolic syndrome; NEFA, nonesterified fatty acid; PEPCK, phosphoenolpyruvate carboxykinase; PP2, protein phosphatase 2; PPase, protein phosphatase; PTP1B, protein tyrosine phosphatase 1B; PTT, pyruvate tolerance test; ROS, reactive oxygen species; SOD1, Cu,Zn-superoxide dismutase; SREBP, sterol-regulatory binding protein; TC, total cholesterol; TG, total triglycerides

* Corresponding author. Fax: +1 607 255 9829.
*E-mail address:* XL20@cornell.edu (X.G. Lei).

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glucose homeostasis. Although body glucose and lipid metabolism is well coordinated, and liver represents a major organ for these metabolic events in mice [16], it is unclear if the previously observed abnormal body weight and glucose status in the SOD1$^{-/-}$ mice [15] concur with altered lipid profiles and dysregulation of key factors for glycolysis, gluconeogenesis, and lipogenesis.

As a master regulator of energy metabolism, AMP-activated protein kinase (AMPK) upregulates the major energy-generating pathways (glycolysis), but downregulates gluconeogenesis [17]. Phosphoenolpyruvate carboxykinase (PEPCK) is the rate-limiting enzyme in gluconeogenesis [18], whereas glucokinase (GK) plays a key role in glucose oxidation (glycolysis) [19]. Meanwhile, sterol-regulatory binding protein 1c (SREBP1c) and sterol-regulatory binding protein 2 (SREBP2) are transcription factors regulating fatty acid, triglyceride, and cholesterol synthesis in liver [20]. Many studies have demonstrated that SREBP1c mediates positive actions of insulin on glycolytic and lipogenic genes [21,22]. Mueller et al. [23] have suggested that activation of protein tyrosine phosphatase 1B (PTP1B) stimulates the lipogenic pathway by inducing SREBP1c expression. Choi et al. [24] have shown that AMPK cascades are highly sensitive to oxidative stress, and the β1 and β2 subunits of AMPK are transcriptionally upregulated via a p53-dependent signal pathway [25]. Nevertheless, it remains unclear as to how these key factors are affected by changes in intracellular superoxide and hydrogen peroxide status associated with the SOD1 and GPX1 knockout. Theoretically, ROS or oxidative stress activates c-Jun $NH_2$-terminal kinase (JNK2) phosphorylation, which can be inactivated by protein phosphatases (PPase's) [26,27]. Protein phosphatase 2A (PP2A) is known to be specific for dephosphorylation of PPase's and another important enzyme, fructose-2,6-bisphosphatase, in controlling hepatic glycolysis [28]. In addition, oxidative stress enhances both the activity and the phosphorylation of neutral sphingomyelinase 2 (nSMase2), which is bound directly by the phosphatase calcineurin (also known as PP2B), which acts as an on/off switch for nSMase2 phosphorylation in the presence/absence of oxidative stress [29]. Therefore, it is confirmed that PP2B is relevant to oxidative stress. Furthermore, PP2B may be involved in regulating the lipogenesis pathway [30]. Thus, it is fascinating to find out if the metabolic effects of SOD1 and GPX1 knockout are initiated at these key stress and phosphorylation-related signal proteins.

Therefore, our objectives for this study were to determine: (1) if hepatic activities of glycolysis and gluconeogenesis and profiles of lipids were affected differently by GPX1 and SOD1 knockout and (2) how the resultant metabolic phenotypes were derived from dysregulation of key signal and/or functional proteins involved in glycolysis, gluconeogenesis, and lipogenesis associated with the presumed changes in intracellular ROS status. Our results indicate that knockout of SOD1 impaired glucose homeostasis through the p53–AMPK pathway and lipid metabolism via the PTP1B–SREBP1 pathway. In contrast, knockout of GPX1 showed little effect on those pathways. Thus, we reveal a distinct difference in the physiological function and the underlying mechanism between these two major antioxidant enzymes in carbohydrate and lipid metabolism.

## Materials and methods

### *Chemicals and antibodies*

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise indicated. The following antibodies were used: SREBP1, SREBP2, JNK2, PP2B, and p53 (Santa Cruz Biotechnology, Santa Cruz, CA, USA); PTP1B (Abgent Primary Antibody Co., San Diego, CA, USA); AMPKα1 and phospho-AMPKα1 (Thr172) (Upstate Biotechnology, Lake Placid, NY, USA); anti-rabbit IgG (Bio-Rad Laboratories, Hercules, CA, USA); and anti-mouse IgG (Pierce Chemical Co., Rockford, IL, USA).

### *Animals and pyruvate tolerance test*

Our experiments were approved by the Institutional Animal Care and Use Committee at Cornell University and were conducted in accordance with the National Institutes of Health guidelines for animal care. The GPX1$^{-/-}$ and SOD1$^{-/-}$ mice and their wild-type (WT) littermates were generated from C57BL/6 mice [31,32]. All experimental mice ($n$=6 or 7 per genotype) were male and 6 months of age and were fed a Se-adequate (0.4 mg/kg) Torula yeast–sucrose diet [33]. The mice were given free access to food and distilled water and housed in shoebox cages in a constant-temperature (22 °C) room with a 12-h light/dark cycle. For the pyruvate tolerance test (PTT), mice were fasted (overnight) for 18 h and then given an ip injection of sodium pyruvate at 2 g/kg body wt (dissolved in phosphate-buffered saline (PBS)) [34]. Blood glucose concentrations were measured using a glucometer (Bayer, Elkhart, IN, USA) via tail bleeding at 0 to 180 min after the injection.

### *Liver sample collection and biochemical analyses*

Mice were fasted (overnight) for 8 h and killed as previously described to collect liver samples [35]. To extract lipid for the assay of total cholesterol (TC), total triglycerides (TG), and non-esterified fatty acid (NEFA), a total of 50 mg of liver was mixed with 0.5 ml of PBS and homogenized (Polytron PT3100; Brinkmann Instruments, Littau, Switzerland). After the homogenate was extracted with chloroform:methanol (5:2.5 ml) and centrifuged, the lower layer was transferred into a glass tube, dried under nitrogen gas, and dissolved in 200 ml of ethanol containing 1% Triton. Concentrations of TC, TG, and NEFA in the ethanol solution were measured using kits (Wako Chemicals, Richmond, VA, USA) [36]. Liver glycogen content was measured using a spectrophotometric assay [37].

### *Enzyme activity assays*

Hepatic activity of PEPCK was determined using a spectrophotometric assay as described by Wimonwatwatee et al. [38]. A unit of PEPCK activity is defined as 1 μmol NADH oxidized per minute per milligram protein. Glucokinase activity was measured spectrophotometrically in a glucose-6-phosphate dehydrogenase-driven NAD(P)H fluorescence assay at 340 nm with the following modifications [39]. Mouse liver (50 mg) was homogenized in 500 μl buffer containing 5 mM dithiothreitol, 5 mM EDTA, and 150 mM KCl. After the homogenate was centrifuged at 12,000$g$ for 10 min, the supernatant was used for glucokinase determination. The reaction mixture (200 μl) (15 μl sample) contained 250 mM Tris–HCl, pH 7.50, 750 mM glucose, 1 M KCl, 75 mM ATP, 75 mM $MgSO_4$, 7.5 mM NADP, 40 U glucose-6-P dehydrogenase/ml. A unit of enzyme activity is defined as the amount of glucokinase that catalyzes the formation of 1 μmol glucose-6-phosphate per minute at 30 °C. Total PPase and PP2A activities were measured using $p$-nitrophenol phosphate as substrate and okadaic acid as an inhibitor at 405 nm according to the method of duBell et al. [40]. One unit of total PPase activity is defined as the amount of enzyme providing the formation of 1 μmol $p$-nitrophenol per minute. Protein concentration was estimated using a BCA protein assay kit (Pierce Chemical Co.).

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