Published in final edited form as:

Free Radic Biol Med. 2017 February ; 103: 48–56. doi:10.1016/j.freeradbiomed.2016.12.011.

Catalase Deletion Promotes PreDiabetic Phenotype in Mice

Claire Heit^a, Stephanie Marshall^b, Surrendra Singh^b, Xiaoqing Yu^c, Georgia Charkoftaki^b, Hongyu Zhao^c, David J. Orlicky^d, Kristofer S Fritz^a, David C. Thompson^e, and Vasilis Vasiliou^b

^aDepartment of Pharmaceutical Sciences, School of Pharmacy, University of Colorado Denver Anschutz Medical Campus, 12850 East Montview Boulevard, Aurora, CO 80045, USA

^bDepartment of Environmental Health Services, Yale School of Public Health, Yale University, New Haven CT 06520-8034

^cDepartment of Biostatistics, Yale School of Public Health, Yale University, New Haven CT 06520 Hongyu Zhao, PhD Professor of Public Health (Biostatistics), of Genetics and of Statistics

^dDepartment of Pathology, School of Medicine University of Colorado Anschutz Medical Campus, Aurora, CO, United States of America

^eDepartment of Clinical Pharmacy, School of Pharmacy, University of Colorado Anschutz Medical Campus, 12850 East Montview Boulevard, Aurora, CO 80045, USA

Abstract

Hydrogen peroxide is produced endogenously and can be toxic to living organisms by inducing oxidative stress and cell damage. However, it has also been identified as a signal transduction molecule. By metabolizing hydrogen peroxide, catalase protects cells and tissues against oxidative damage and may also influence signal transduction mechanisms. Studies suggest that acatalasemic individuals (i.e., those with very low catalase activity) have a higher risk for the development of diabetes. We now report catalase knockout (*Cat*^{-/-}) mice, when fed a normal (6.5% lipid) chow, exhibit an obese phenotype that manifests as an increase in body weight that becomes more pronounced with age. The mice demonstrate altered hepatic and muscle lipid deposition, as well as increases in serum and hepatic triglycerides (TGs), and increased hepatic transcription and protein expression of PPAR γ . Liver morphology revealed steatosis with inflammation. *Cat*^{-/-} mice also exhibited pancreatic morphological changes that correlated with impaired glucose tolerance and increased fasting serum insulin levels, conditions consistent with pre-diabetic status. RNA-seq analyses revealed a differential expression of pathways and genes in *Cat*^{-/-} mice, many of which are related to metabolic syndrome, diabetes, and obesity, such as *Pparg* and *Cidec*.

Corresponding author: Vasilis Vasiliou, PhD, Department of Environmental Health Sciences, Yale School of Public Health, 60 College St, New Haven, CT 06250, Tel: 203 737 8094; Fax: 203 724 6023; vasilis.vasiliou@yale.edu.

Author contributions: CH, SM, XY, GC and DO (acquisition, analysis and interpretation of data), CH (study design and preparation of manuscript), SM, XY and KF (study design and critical revision of the manuscript), SM (data interpretation and preparation of the manuscript), DT (data analysis and interpretation, and preparation of the manuscript), VV (study design, interpretation of data, critical revision of the manuscript, obtaining funding, and study supervision).

Conflict of interest: The authors declare that they have no conflict of interest with the contents of the article.

In conclusion, the results of the present study show mice devoid of catalase develop an obese, pre-diabetic phenotype and provide compelling evidence for catalase (or its products) being integral in metabolic regulation.

Keywords

Catalase; obesity; metabolism; steatosis; diabetes

Introduction

Alterations in redox balance are found in numerous pathologies. These often occur as a result of increased reactive oxygen species (ROS) or decreased antioxidant defense systems. Hydrogen peroxide, an important ROS, is produced endogenously and historically has been perceived to be deleterious by promoting oxidant damage to cells [1]. Well-known for its role in metabolizing hydrogen peroxide to water and oxygen, catalase prevents cellular oxidant damage by removing hydrogen peroxide. In the absence of catalase, hydrogen peroxide can be metabolized by other antioxidant enzymes, such as glutathione peroxidase (GPX), or it can form hydroxyl radicals through Fenton reactions [2]. These hydroxyl radicals can initiate a free radical cascade and thereby damage numerous macromolecules, such as lipids (peroxidation), and promote amino acid oxidation. Catalase, normally localized to the peroxisome where beta oxidation reactions produce hydrogen peroxide [3], functions as an antioxidant by catalyzing the conversion of hydrogen peroxide to oxygen and water. Several studies have emphasized the correlation between oxidative stress, obesity, and insulin resistance in type 2 diabetes [4-7]. In addition to its well-established role as a oxidant, hydrogen peroxide can act as a physiological signal transduction molecule [8]. Catalase, by influencing cellular levels of hydrogen peroxide, would be anticipated to also regulate hydrogen peroxide-dependent signal transduction pathways.

Acatalasemia is a rare genetic deficiency in humans that involves severe reductions in catalase activity. Given the important role played by catalase in detoxifying hydrogen peroxide, it would be predicted that catalase-deficient populations (acatalasemic and hypocatalasemic) would suffer from overt oxidative damage-related disease; however, this has not proven to be the case. Acatalasemia in humans has been considered to be an asymptomatic disorder [9] and catalase deficient mice are described as being phenotypically normal [10]. Nevertheless, more recent epidemiological studies show acatalasemic human subjects to be at an increased risk for developing numerous pathologies including altered lipid and carbohydrate metabolism [11] and type 2 diabetes [12, 13].

Ho and colleagues created global catalase knockout (*Car*^{-/-}) mice by gene targeting of intron 4 and exon 5 [10]. These mice have a diminished rate of removal of hydrogen peroxide and thus are more susceptible to oxidant tissue injury [10]. Catalase-deficient mice, while appearing to be phenotypically normal [10, 11], are more vulnerable to induction of diabetes by alloxan [14] and to renal injury associated with streptozotocin-induced diabetes [13]. This is thought to be caused by accumulating ROS damaging pancreatic beta cells of the islets of Langerhans and thereby reducing insulin secretion [15, 16]. Low levels of protective

antioxidants in beta cells [17, 18] would also be a factor contributing to the beta cell vulnerability. Several lines of evidence support a role for hydrogen peroxide in ROS-induced beta cell toxicity. For example, sequestration of hydrogen peroxide by alpha lipoic acid attenuates the toxicity [19, 20], while reduced expression of catalase mRNA and protein may promote toxicity [21-23] by allowing the accumulation of hydrogen peroxide and thereby enhancing ROS-induced cellular damage.

Genes involved in metabolism, such as transcription factors, can also be influenced by cellular hydrogen peroxide. Peroxisome proliferator-activated receptors (PPARs) are transcription factors that play an important role in obesity and insulin sensitivity [24]. Agonists of PPARs, thiazolidinediones (TZDs), have been used to treat diabetic insulin resistance [24, 25]. Although the exact mechanism remains to be established with certainty, TZDs act on PPAR γ which alters transcription of numerous genes, ultimately leading to decreased insulin resistance and increased adipocyte lipid storage. Thiazolidinediones also attenuate ROS-induced beta cell damage through a mechanism involving induction of catalase [26]. In addition to influencing the effects of insulin, PPAR γ activation increases hepatic lipogenesis, and blood and tissue lipid accumulation [27-29].

The present study uses the *Cat*^{-/-} mouse model to examine the role of catalase in lipid dysfunction and metabolic regulation. It was predicted that catalase deficiency would adversely affect lipid trafficking and glucose homeostasis by promoting the accumulation of hydrogen peroxide and thereby eliciting oxidant damage and/or amplified signal transduction pathways.

Materials and Methods

Animals

All procedures involving animals were approved by the institutional Animal Care and Use Committee of the University of Colorado and were performed in accordance with published National Institute of Health guidelines. The catalase knockout (*Cat*^{-/-}) mice were generated as previously described in the C57BL/6 background [10] and obtained from Dr. Ho (Wayne State, MI).

Male C57BL/6 wild-type (WT) and *Cat*^{-/-} mice were housed in groups (3-5 per group) with water and standard chow (Teklad 2920x: 6.5% fat, 19.1% protein, 47.0% carbohydrate) diet available *ad libitum*. Mice were weighed weekly and all but one *Cat*^{-/-} mouse survived the duration of the study. Necropsy revealed no overt cause of death for the non-surviving mouse. Upon completion of the study, mice were euthanized by CO₂ followed by decapitation. Tissues were excised, weighed and fixed, or flash frozen for biochemical analysis.

Protein expression and carbonylation

Frozen liver tissues were homogenized on ice in lysis buffer containing phosphatase and protease inhibitors. Proteins were prepared for SDS PAGE as previously described [30]. Protein concentrations were determined using a BCA Protein Assay (Pierce, Rockford, IL). Proteins (24 μ g) were separated using a 10% SDS gel and subsequently transferred to a

PVDF membrane. Membranes were blocked for 60 min with Tris-buffered saline solution containing 1% Tween-20 (TBST) and 5% non-fat dry milk, and probed overnight with rabbit primary antibodies (catalase: 1:5000, Sigma; PPAR γ : 1:1000, Novus Biologicals, CO). A horseradish peroxidase-conjugated goat secondary antibody (Jackson Immune Research, PA) was then applied and membranes developed using ECL-Plus Reagent (GE Healthcare, UK). Chemiluminescence was visualized using either film or a Storm 860 scanner (Molecular Dynamics, Sunnyvale, CA). Biotin hydrazide detection of carbonylated proteins was conducted as previously described [31]. Briefly, liver homogenates were treated with 5mM biotin hydrazide (BH; Pierce, Rockford, IL) for 2 h in the dark (corresponding untreated controls were incubated in dark without addition of BH). Proteins were then loaded onto a 10% SDS gel and then transferred to a PVDF membrane. A rabbit anti-biotin antibody (GeneTex, Irvine, CA) was applied and imaged using the Storm 860 scanner (Molecular Dynamics, Sunnyvale, CA). Densitometric analyses were performed using ImageJ software (NIH, USA) and normalized to total protein content (Ponceau).

Biochemical analyses

The liver was removed *en bloc* from 12-15 mo old mice and flash frozen in liquid nitrogen. One lobe was excised, weighed and homogenized in buffer. Liver triglycerides were measured in a 2:1 chloroform:methanol extract of liver homogenate using a triglyceride-SL kit (Seikesui, Japan). Serum was derived from blood drawn from the inferior vena cava or submandibular face vein. Triglycerides and alanine transferase (ALT) were measured using commercially available kits (triglyceride-SL, Alanine Aminotransferase-SL assay, Seikesui Assay Kit, Japan). Fasting serum insulin was collected from mice at 5 mo old, before the body weights were different and 8 mo old, at which time the weights were significantly increased (Fig. 1C). Serum insulin was determined using a commercially-available kit for mouse insulin (Crystal Chem, IL, USA).

Glucose Tolerance Test (GTT)

Nine mo old WT and *Car*^{-/-} mice were fasted overnight with water available *ad libitum*. Mice were anesthetized by isoflurane inhalation. The tail was then nicked to allow initial collection (t = 0) of blood from the tail vein for measurement of fasting blood glucose. Fifteen % (w/v) D-glucose, prepared by dissolution in tap water and passage of the solution through a sterile filter, was administered intraperitoneally (1.5 g/kg). Blood was then sampled from the tail vein 15, 30, 60 and 120 min thereafter and glucose levels were determined using a glucometer (OneTouch Ultra, One Touch). The area under the curve (AUC) of the blood glucose concentration–time curve was calculated using Graphpad Prism V 6.0 (La Jolla, CA).

mRNA determination (RNA-seq)

The liver was excised from 2 mo old mice (n=3) and flash frozen in liquid nitrogen. RNA was isolated the same day using a commercially-available kit (Qiagen, RNeasy kit). RNA-seq was run on two of the samples from each genotype and through biostatistical analysis detailed below. Data presented is combined from two animals. Mice of this age were selected to allow determination of RNA changes that might underlie latter physiological changes.

Quality control—Total RNA quality was determined spectrophotometrically (Nanodrop, Thermo Fisher Scientific Inc., USA) using the A_{260}/A_{280} and A_{260}/A_{230} ratios. RNA integrity was verified by the relative abundance of 18S and 28S subunits of ribosomal RNA (Agilent 2100 Bioanalyzer).

Library preparation—RNA samples (n=2) were prepared using the Illumina TruSeq RNA Library Preparation Kit v2. Messenger RNA was purified from 500ng of total RNA using oligo-dT beads and sheared by incubation at 94°C. Following first-strand synthesis with random primers, second strand synthesis was performed with dUTP for generating strand-specific sequencing libraries. The cDNA library was then end-repaired and A-tailed, adapters were ligated and second-strand digestion was performed using uracil DNA glycosylase. Indexed libraries were quantified by qRT-PCR using a commercially-available kit (KAPA Biosystems, Wilmington, MA). The insert size distribution was determined using the Agilent Bioanalyzer High Sensitivity DNA Chip. Samples with a concentration of 0.5 ng/μl were used for sequencing.

Flow cell preparation and sequencing—Sample concentrations were normalized to 2 nM and loaded onto rapid or high-output flow cells (Illumina, San Diego, CA) at a concentration that yielded 150-250 million passing filter clusters per lane. Samples were sequenced using 75 bp single or paired-end sequencing (Illumina HiSeq 2500) according to Illumina protocols. Data generated during sequencing runs were transferred to the Yale Center for Genomic Analysis (YCGA) high-performance computing cluster. A positive control (prepared bacteriophage Phi X library provided by Illumina) was spiked into every lane at a concentration of 0.3% to monitor sequencing quality in real time.

Data analysis and storage—Signal intensities were converted to individual base calls during a run using Real Time Analysis (RTA) software (Illumina). Primary sample analysis de-multiplexing and alignment to the human genome was performed using CASAVA 1.8.2 software suite (Illumina, San Diego, CA). The raw RNA-sequencing reads were first assessed for quality using FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). FASTX-toolkit [32] was then used to trim off adaptor sequences and low quality bases. Reads passing the quality control were aligned to the Ensembl annotation of mouse genome reference sequence GRCm38 by TopHat 2 [33] and Bowtie [34] alignment engines. Reads mapped to multiple locations were discarded from further analysis. Raw gene counts (gene expression levels) were established by HTSeq package [35] with the default union-counting mode, and normalized using the TMM method in edgeR package [36]. A negative binomial generalized linear model implemented in edgeR [37-40] was used to determine differentially-expressed genes with multiple experimental factors accounted for. Genes from *Cat*^{-/-} mice with fold-change (> 2 or < 0.5) and false discovery rate (FDR)-controlled (P < 0.05) were considered differentially expressed relative to WT mice.

Histology

Liver and pancreas were excised *en bloc* from 12-15 mo old mice and weighed. Visceral and subcutaneous white adipose tissue (WAT) was crudely collected from each mouse and weighed. Sections of liver lobes, and adipose and muscle tissue, and the entire pancreas

were collected, fixed in 10% neutral buffered formalin and embedded in paraffin for histological examination. Slides were cut and prepared for hematoxylin and eosin (H&E) staining by the UCD Research Histology Shared Resource. Brunt scoring for liver damage was adapted for use in the mouse as previously described [41, 42]. Briefly, the extent of steatosis, the presence of inflammatory cells and foci, features of hepatocyte injury, markers of tissue response, and Picrosirius Red-detectable collagen were determined by cell morphology and staining by a trained histopathologist; the extent of each was quantitated by the histopathologist blinded to the origin of the slides. Histologic images were captured on an Olympus BX51 microscope equipped with a 4MP Macrofire digital camera (Optronics) using the PictureFrame Application 2.3 (Optronics). Cross-polarized light was used to enhance visualization of Picrosirius Red-stained images as previously described. Briefly, slides were imaged with polarized light in a tiling fashion across the whole tissue and analysis of the images by the 3I program slidebook (3I, Denver, Colorado). Islet areas (% of WT) were determined from H&E-stained histological images using ImageJ software (NIH, USA).

Statistical analyses

AUC differences were identified by Student's unpaired t-test or two-way ANOVA. Biochemical and histological comparisons between *Cat*^{-/-} and WT mice were performed using a Student's unpaired t-test. Body weight differences were determined by two-way ANOVA with differences at each time point being analyzed using a Student's unpaired t-test. Statistical comparisons were conducted using Graphpad Prism v 6.0 (La Jolla, CA). Statistical significance was defined as $P < 0.05$.

Results

Catalase knockout, oxidative stress

The *Cat*^{-/-} knockout genotype was confirmed by tail snip genotyping (data not shown), the absence of hepatic expression of catalase protein (Fig. 1A) and RNA-seq analyses (Table 1). RTPCR was not conducted to verify RNA-seq analysis results due to altered internal control genes in *Cat*^{-/-} mice. While mRNA for catalase was shown to be repressed, the mRNA for other peroxide-metabolizing enzymes, including glutathione peroxidase (*Gpx*) and peroxiredoxin (*Prx*), were unchanged in the *Cat*^{-/-} mice (data not shown). Protein carbonylation, a marker of the action of ROS on proteins, was observed in *Cat*^{-/-} mice (Fig. 1B).

Catalase knockout mice fed standard chow diet exhibited increased body weight relative to their corresponding WT controls beginning after 6 mo of age (Fig. 1C). In spite of the increased body weight, the WAT weight-to-total body weight ratio in *Cat*^{-/-} mice was not different from WT mice (Table 2). Nevertheless, histological analysis revealed adipose tissue from *Cat*^{-/-} mice to contain hypertrophic adipocytes as well as an increased number of macrophages surrounding some of the adipocytes (crown-like structures) relative to their WT counterparts (Fig. 1D).

Significant hepatic changes were induced by the absence of catalase. The liver weight of *Cat*^{-/-} mice was increased relative to WT controls (Table 2). Morphologically, the livers of *Cat*^{-/-} mice had a lighter yellow color than WT control (data not shown) and histological analysis revealed lipid accumulation in the livers of *Cat*^{-/-} mice (Fig. 2A) consistent with steatosis. *Cat*^{-/-} mice also had a higher incidence of more advanced macrosteatosis (10-33%) and microsteatosis (83%) (Table 3). Inflammation (manifesting as foci of inflammatory cells and the presence of lipogranulomas) and liver cell injury (ballooning degeneration) were not observed in the livers of WT mice but were present in at least 33% of the livers of *Cat*^{-/-} mice (Table 3). Liver fibrosis was not observed in any animals. Elevated levels of triglycerides were observed in the serum and liver of *Cat*^{-/-} mice relative to their WT counterparts (Table 2). A trend for increased serum ALT was also observed in the 12 mo old *Cat*^{-/-} mice but it failed to reach significance due to large variability (*P* = 0.2) (Table 2). Expression of the transcription factor, PPAR γ , was increased in the livers of *Cat*^{-/-} mice (Fig. 2B).

There were a total of 1,102 up-regulated transcripts and 1,351 down-regulated transcripts in the livers of *Cat*^{-/-} mice. The five most up-regulated transcripts include Cell Death-Inducing DFFA-Like Effector C (*Cidec*, 124-fold), Transmembrane protein 28 (*Tmem28*, 119-fold), Oxysterol Binding Protein-Like 3 (*Osbpl3*, 33-fold), Complement factor D, (corresponding to human adipsin) (*Cfd*, 31-fold), and monoacylglycerol acyltransferase (*Mogat1*, 26-fold). Because of the indication of lipid dysregulation seen in histology and up-regulated transcripts, we evaluated genes previously reported to be involved in obesity [33]. These were found to be differentially expressed in *Cat*^{-/-} mice (Table 4). Leptin (*Lepr* 0.37-fold change) and insulin signaling/sensitivity (*Irs2* 0.29-fold change) were decreased while *Irs3* was increased (3.1-fold change). Transcripts involved in free fatty acid metabolism, specifically *Adra1a* and *Lipg* (3.2- and 2.4-fold change, respectively), were increased while *Adrb2* and *Lpl* were decreased (0.4- and 0.3-fold, respectively). Transcripts involved in lipid biosynthesis, such as *Fasn* (2.7-fold change) and *Scd1-4* (2.0-2.5-fold change), were also increased in *Cat*^{-/-} mice. Transcripts involved in lipid transport, specifically importing genes *Cd36* (4.9-fold change) and *Slc2a4* (4.4-fold change), were increased.

Fasting blood glucose levels in *Cat*^{-/-} mice were comparable to WT mice (Table 5). Nevertheless, *Cat*^{-/-} mice showed impaired tolerance to a bolus dose of glucose such that higher blood glucose levels than WT mice occurred at 15 and 30 min, and with a trend at 60 min (*P* = 0.06) (Fig. 3A). Relatedly, the AUC for the glucose tolerance test curve was greater for *Cat*^{-/-} mice than WT mice (Fig. 3B). Histological analysis of the pancreas showed a larger islet area in *Cat*^{-/-} mice than in WT mice (Fig. 3C, D). Higher fasting insulin was measured in the serum of the *Cat*^{-/-} mice (Table 5). Intermuscular adipose tissue was found in the gastrocnemius skeletal muscle of *Cat*^{-/-} mice but not WT mice (Fig. 3E).

Discussion

The genesis of this study emanated from our observation that catalase knockout (*Cat*^{-/-}) mice gained weight at a faster rate later in their life (i.e., after 6 mo of age) while maintained on a normal low fat chow diet. For example, at 8 mo of age, *Cat*^{-/-} mice weighed 23% more than wild-type mice. This is less than the weight gain observed in two established mouse models

of obesity, *ob/ob* and *db/db*, in which the increases are approximately 200 and 60% (respectively) more than wild-type [43-45].

Initial experiments revealed the weight gain in *Cat*^{-/-} mice to be unrelated to the accumulation of white adipose tissue. However, the increase in intermuscular adipose tissue observed in *Cat*^{-/-} mice may have contributed to the weight gain. Although the amount of white adipose tissue did not change in *Cat*^{-/-} mice, the nature of this tissue differed from wild-type mice in containing hypertrophic and irregular adipocytes, and increased numbers of crown-like structures. Such ultrastructural changes, when taken together with the observed elevated levels of serum triglycerides, suggest that an absence of catalase promotes alterations in lipid mobilization and utilization that favor excess circulating lipids. This is of particular interest as the mice were fed a standard chow diet which is low fat, but high carbohydrate. Notably, crown-like structures, reflective of macrophages surrounding dying or dead adipocytes, are commonly observed in the adipose tissue of *ob/ob* and *db/db* mice and are thought to be associated with insulin resistance [46]. A recent study showed higher levels of plasma free fatty acids and triglycerides in *Cat*^{-/-} mice [47], reaffirming a potential role for catalase in lipid mobilization. The same study also revealed increased numbers of crown-like structures in white adipose tissue in *Cat*^{-/-} mice, but only under conditions of a high fat diet.

Catalase deficiency significantly impacted the liver and its function. Specifically, the liver of *Cat*^{-/-} mice weighed more, contained higher levels of triglycerides, and exhibited histopathological changes associated with hepatosteatosis, including microsteatosis and macrosteatosis. The hepatocyte injury (ballooning degeneration) and inflammation is indicative of the development of steatohepatitis in the *Cat*^{-/-} mice, i.e., a progression from non-alcoholic fatty liver disease (NAFLD) to non-alcoholic steatohepatitis (NASH). These data lend further support to the notion that loss of catalase adversely affects lipid homeostasis. Hepatic mRNA expression analyses demonstrated changes that would be predicted to affect fatty acid handling by the body, including increases in adipsin (*Cfd*), cell death inducing DFFA-like effector C (*Cidec*), oxysterol binding protein-like 3 (*Oshpl3*), protein phosphatase 1 regulatory subunit 3G (*Ppp1r3g*), monoacylglycerol acyltransferase (*Mogat1*), fatty acid translocase (*Cd36*), fatty acid synthase (*Fasn*), endothelial lipase (*Lipg*), stearoyl-coA desaturases (*Scd1*, *Scd2*, *Scd3*, *Scd4*), alpha_{1A} adrenoceptors (*Adra1a*) and decreases in mRNA expression of lipoprotein lipase (*Lpl*), beta₂ adrenoceptors (*Adrb2*) and 3-hydroxy-3-methylglutaryl-coA reductase (*Hmgcr*).

Macrophage accumulation in white adipose tissue has been correlated with insulin resistance and hyperinsulinemia in mice [48]. Under fasting conditions, the *Cat*^{-/-} mice exhibited elevated levels of insulin (hyperinsulinemia) that were comparable to those observed previously in *db/db* mice [49]. Histological analysis of the pancreas of *Cat*^{-/-} mice revealed larger islets of Langerhans, a pathophysiological change previously documented in obese (*ob/ob*) and diabetic (*db/db*) mice [50]. Increases in beta cell mass and higher levels of fasting plasma insulin have also been noted in obese h-IAPP transgenic mice [51]. Fasting blood glucose levels in *Cat*^{-/-} mice were not different from wild-type mice, suggesting that the *Cat*^{-/-} mice were not diabetic but were more resistant to insulin than their wild-type counterparts. Intermuscular adipose tissue has been shown to positively correlate to insulin

resistance and higher fasting insulin [52, 53]. Increases in pancreatic beta cell mass and insulin secretion have been proposed to represent pathophysiological adaptations to chronic insulin resistance and are similar what is thought to occur during metabolic syndrome [51] in humans.

The reduced capacity of *Cat*^{-/-} mice to buffer glucose (manifesting as elevated peak blood glucose levels and an increased area under the glucose tolerance test curve in response to a bolus administration) indicates that the absence of catalase adversely affects glucose homeostasis and renders mice in a pre-diabetic state. Changes in transcriptional expression for facilitated glucose transporter 4 (*Glut4*) and protein phosphatase 1 regulatory subunit 3G would be expected to contribute to these effects. These results in *Cat*^{-/-} mice align with those in hypocatalasemic human subjects with diminished catalase activity who have a higher risk ($\approx 13\%$) of developing diabetes [54] and in acatalasemic mutant mice that are more vulnerable to induction of diabetes by alloxan [14]. RNA-Seq experiments in liver tissue from *Cat*^{-/-} mice revealed changes that would influence the actions of insulin, including down-regulation of insulin-like growth factor binding protein 1 (*Igfbp1*) and insulin receptor substrate 2 (*Irs2*), and up-regulation of insulin receptor substrate 3 (*Irs3*). *IRS2* has previously been shown to control glucose homeostasis and be involved in insulin resistance [55] as *Irs2*^{-/-} mice develop type 2 diabetes despite compensatory changes in *IRS3* [56]. Insulin inhibits insulin-like growth factor binding protein 1 gene expression in the liver [57]. Accordingly, the elevated levels of serum insulin in the *Cat*^{-/-} mice would be predicted to decrease *Igfbp1* mRNA.

Peroxisome proliferator-activated receptors (PPARs) are transcription factors that play an important role in obesity and insulin sensitivity [24]. Agonists of PPAR γ have been used clinically to reduce insulin resistance and treat type 2 diabetes [58]; A common contention is that they do so by increasing the quantity of adipose tissue which then acts as a “sink” for the excess glucose [59]. Liver transcripts for and protein levels of PPAR γ were elevated in *Cat*^{-/-} mice. PPAR γ promotes elevated hepatic triglyceride levels and fat accumulation, and steatosis by increasing expression of genes that promote lipogenesis, lipid transport and beta-oxidation, such as *adipsin*, *cd36*, *fsp27* (*cidec*) and *mogat1* [27, 60-62]. Hence, many of the transcript changes observed in the liver of *Cat*^{-/-} mice could involve PPAR γ .

The effects of catalase absence on lipid and glucose homeostasis would be predicted to relate to the accumulation of hydrogen peroxide and subsequent amplification of hydrogen peroxide-dependent signal transduction pathways or oxidant damage. Ho and colleagues, the originators of the mice used in the present study, showed increased hydrogen peroxide production by tissue from *Cat*^{-/-} mice [10]. Park and colleagues demonstrated elevated nitrotyrosine expression in epididymal white adipose tissue of *Cat*^{-/-} mice [47]. Reactive oxygen species are abundantly produced in obesity and metabolic syndrome [5] and adipose tissue proteins of obese patients have been found to be more carbonylated (a marker of oxidative stress) [63]. Elevated levels of carbonylated proteins in the liver of *Cat*^{-/-} mice indicate oxidative stress was occurring in the absence of catalase.

Hydrogen peroxide plays an important role in insulin-producing cells, insulin signaling, and insulin resistance. It alters adipokine signaling pathways, and genes involved in metabolic

function, such as transcription factors, inflammation markers, and insulin receptor signaling molecules, and can be toxic to beta cells of the islets of Langerhans [17, 64-67] Beta cells have relatively low levels of antioxidant enzymes [68] and catalase is important for protecting these cells from the toxic effects of ROS [5]. Consistent with such a protective role is the demonstration that acatalasemic mice are more vulnerable to induction of diabetes by alloxan, a treatment that induces ROS formation in beta cells [14]. In the present study, the absence of catalase did not appear to be toxic to beta cells because the islets of Langerhans were increased (in size and number) in *Cat*^{-/-} mice, as were serum levels of insulin.

The present study has demonstrated the absence of catalase leads to insulin resistance in mice. Twin studies in humans have strongly implicated diet as a root cause of insulin resistance [69]. For example, the consumption of fructose, a commonly-used sweetener and food additive, is strongly linked to insulin resistance [70]. This same sugar has been shown to lower hepatic catalase mRNA and activity in experimental animals [21, 71]. These data support the intriguing possibility that modulation of catalase by fructose could contribute to the development of insulin resistance induced by this sugar.

In summary, the results of the present study show increased oxidative stress and increased body weight in *Cat*^{-/-} mice. There is excess lipid accumulation in adipocytes and hepatocytes, as well as in skeletal muscle. Excess lipid in the liver is accompanied by inflammation and damage, and transcriptional changes in genes involved in lipid synthesis (*Fasn*) and transport (*Cd36*), and insulin sensitivity (*Irs2*, *Pparg*). Glucose intolerance occurred in *Cat*^{-/-} mice with pancreatic changes manifesting as more numerous enlarged islets of Langerhans and higher fasting serum insulin levels. Together, these results provide compelling evidence for catalase (or its products) being involved in insulin resistance and development of a pre-diabetic state.

Acknowledgments

We would like to thank Dr. Ying Chen of Yale School of Public Health and Peter Harris of University of Colorado Department of Pharmaceutical Science for their assistance, expertise and support with this project. This work is supported in part by NIH grants AA022057 (VV), AA021724 (VV). SM was supported by the NIAAA F32 FAA023699A. UO1 IU01AA021724-01A1 (VV), Cancer Center Support Grant (P30CA046934)

References

1. Camhi SL, Lee P, Choi AM. The oxidative stress response. *New Horizons Journal*. 1995; 3(2):170–82.
2. Lubos E, Loscalzo J, Handy DE. Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities. *Antioxidant and Redox Signaling*. 2011; 15(7):1957–97.
3. J, A., Alberts, B., Lewis, J., et al. *Molecular Biology of the Cell “Peroxisomes”*. Garland Science; New York:: 2002.
4. Evans JL, Maddux BA, Goldfine ID. The molecular basis for oxidative stress-induced insulin resistance. *Antioxidant and Redox Signaling*. 2005; 7(7-8):1040–52.
5. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I. Increased oxidative stress in obesity and its impact on metabolic syndrome. *Journal of Clinical Investigation*. 2004; 114(12):1752–61. [PubMed: 15599400]

6. Ikemura M, Nishikawa M, Hyoudou K, Kobayashi Y, Yamashita F, Hashida M. Improvement of insulin resistance by removal of systemic hydrogen peroxide by PEGylated catalase in obese mice. *Molecular Pharmaceutics*. 2010; 7(6):2069–76. [PubMed: 21033698]
7. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? *Free Radical Biology and Medicine*. 2010; 49(11):1603–16. [PubMed: 20840865]
8. Groeger G, Quiney C, Cotter TG. Hydrogen peroxide as a cell-survival signaling molecule. *Antioxidant and Redox Signaling*. 2009; 11(11):2655–71.
9. Takahara S, Ogata M. Erythrocyte metabolism against oxidation in Japanese acatalasemia. *Monographs in Human Genetics*. 1978; 10:205–11. [PubMed: 723895]
10. Ho YS, Xiong Y, Ma W, Spector A, Ho DS. Mice lacking catalase develop normally but show differential sensitivity to oxidant tissue injury. *The Journal of Biological Chemistry*. 2004; 279(31):32804–12. [PubMed: 15178682]
11. Goth L, Rass P, Pay A. Catalase enzyme mutations and their association with diseases. *Journal of Molecular Diagnostics*. 2004; 8(3):141–9.
12. Goth L, Nagy T. Inherited catalase deficiency: is it benign or a factor in various age related disorders? *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*. 2013; 753(2):147–54. [PubMed: 24025477]
13. Hwang I, Lee J, Huh JY, Park J, Lee HB, Ho YS, Ha H. Catalase deficiency accelerates diabetic renal injury through peroxisomal dysfunction. *Diabetes*. 2012; 61(3):728–38. [PubMed: 22315314]
14. Kikumoto Y, Sugiyama H, Inoue T, Morinaga H, Takiue K, Kitagawa M, Fukuoka N, Saeki M, Maeshima Y, Wang DH, Ogino K, Masuoka N, Makino H. Sensitization to alloxan-induced diabetes and pancreatic cell apoptosis in acatalasemic mice. *Biochimica et Biophysica Acta*. 2010; 1802(2):240–6. [PubMed: 19883754]
15. Roma LP, Bosqueiro JR, Cunha DA, Carneiro EM, Gurgul-Convey E, Lenzen S, Boschero AC, Souza KL. Protection of insulin-producing cells against toxicity of dexamethasone by catalase overexpression. *Free Radic Biol Med*. 2009; 47(10):1386–93. [PubMed: 19698781]
16. Xu B, Moritz JT, Epstein PN. Overexpression of catalase provides partial protection to transgenic mouse beta cells. *Free Radic Biol Med*. 1999; 27(7-8):830–7. [PubMed: 10515587]
17. May JM, de Haen C. The insulin-like effect of hydrogen peroxide on pathways of lipid synthesis in rat adipocytes. *The Journal of Biological Chemistry*. 1979; 254(18):9017–21. [PubMed: 479177]
18. Veal EA, Day AM, Morgan BA. Hydrogen peroxide sensing and signaling. *Molecular Cell*. 2007; 26(1):1–14. [PubMed: 17434122]
19. Robertson RP, Harmon J, Tran PO, Tanaka Y, Takahashi H. Glucose toxicity in beta-cells: type 2 diabetes, good radicals gone bad, and the glutathione connection. *Diabetes*. 2003; 52(3):581–7. [PubMed: 12606496]
20. Wu L, Nicholson W, Knobel SM, Steffner RJ, May JM, Piston DW, Powers AC. Oxidative stress is a mediator of glucose toxicity in insulin-secreting pancreatic islet cell lines. *The Journal of Biological Chemistry*. 2004; 279(13):12126–34. [PubMed: 14688272]
21. Cavarape A, Feletto F, Mercuri F, Quagliaro L, Daman G, Ceriello A. High-fructose diet decreases catalase mRNA levels in rat tissues. *J Endocrinol Invest*. 2001; 24(11):838–45. [PubMed: 11817707]
22. Maithili Karpaga Selvi N, Sridhar MG, Swaminathan RP, Sripradha R. Curcumin attenuates oxidative stress and activation of redox-sensitive kinases in high fructose- and high-fat-fed male wistar rats. *Scientia Pharmaceutica*. 2015; 83(1):159–75. [PubMed: 26839808]
23. Francini F, Castro MC, Schinella G, Garcia ME, Maiztegui B, Raschia MA, Gagliardino JJ, Massa ML. Changes induced by a fructose-rich diet on hepatic metabolism and the antioxidant system. *Life Sciences*. 2010; 86(25-26):965–71. [PubMed: 20470786]
24. Day C. Thiazolidinediones: a new class of antidiabetic drugs. *Diabetic Medicine*. 1999; 16(3):179–92. [PubMed: 10227562]
25. Rizos CV, Kei A, Elisaf MS. The current role of thiazolidinediones in diabetes management. *Archives of Toxicology*. 2016

26. Chung SS, Kim M, Lee JS, Ahn BY, Jung HS, Lee HM, Park KS. Mechanism for antioxidative effects of thiazolidinediones in pancreatic beta-cells. *American Journal of Physiology Endocrinology and Metabolism*. 2011; 301(5):E912–21. [PubMed: 21846907]
27. Yu S, Matsusue K, Kashireddy P, Cao WQ, Yeldandi V, Yeldandi AV, Rao MS, Gonzalez FJ, Reddy JK. Adipocyte-specific gene expression and adipogenic steatosis in the mouse liver due to peroxisome proliferator-activated receptor gamma1 (PPARgamma1) overexpression. *The Journal of Biological Chemistry*. 2003; 278(1):498–505. [PubMed: 12401792]
28. Bai L, Zhang Y, Xie C, Wang R, Zhao S, Jia Y, Liu E. Overexpression of PPARgamma induces adipogenic steatosis in mouse primary hepatocytes. *Journal of Zhejiang University*. 2016; 45(1): 68–74.
29. Li Q, Chen R, Bie L, Zhao D, Huang C, Hong J. Association of the variants in the PPARG gene and serum lipid levels: a meta-analysis of 74 studies. *Journal of Cellular and Molecular Medicine*. 2015; 19(1):198–209. [PubMed: 25265984]
30. Chen Y, Singh S, Matsumoto A, Manna SK, Abdelmegeed MA, Golla S, Murphy RC, Dong H, Song BJ, Gonzalez FJ, Thompson DC, Vasiliou V. Chronic glutathione depletion confers protection against alcohol-induced steatosis: implication for redox activation of AMP-activated protein kinase pathway. *Scientific Reports*. 2016; 6:29743. [PubMed: 27403993]
31. Grimsrud PA, Xie H, Griffin TJ, Bernlohr DA. Oxidative stress and covalent modification of protein with bioactive aldehydes. *The Journal of Biological Chemistry*. 2008; 283(32):21837–41. [PubMed: 18445586]
32. Pearson WR, Wood T, Zhang Z, Miller W. Comparison of DNA sequences with protein sequences. *Genomics*. 1997; 46(1):24–36. [PubMed: 9403055]
33. Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg S. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biology*. 2013; 14(4):R36. [PubMed: 23618408]
34. Langmead B, Trapnell C, Pop M, Salzberg SL. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biology*. 2009; 10:R25. [PubMed: 19261174]
35. Anders S, Pyl PT, Huber W. HTSeq—a Python framework to work with high-throughput sequencing data. *Bioinformatics*. 2015; 31(2):166–169. [PubMed: 25260700]
36. Robinson MD, Oshlack A. A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biology*. 2010; 11(3):1–9.
37. Robinson MD, Smyth GK. Moderated statistical tests for assessing differences in tag abundance. *Bioinformatics*. 2007; 23:2881–2887. [PubMed: 17881408]
38. Robinson MD, Smyth GK. Small-sample estimation of negative binomial dispersion, with applications to SAGE data. *Biostatistics*. 2008; 9:321–332. [PubMed: 17728317]
39. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*. 2010; 26:139–140. [PubMed: 19910308]
40. Robinson MD. Copy-number-aware differential analysis of quantitative DNA sequencing data. *Genome Research*. 2012; 22:2489–2496. [PubMed: 22879430]
41. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *American Journal of Gastroenterology*. 1999; 94(9):2467–74.
42. Lanaspa MA, Ishimoto T, Li N, Cicerchi C, Orlicky DJ, Ruzyczki P, Rivard C, Inaba S, Roncal-Jimenez CA, Bales ES, Diggie CP, Asipu A, Petrash JM, Kosugi T, Maruyama S, Sanchez-Lozada LG, McManaman JL, Bonthron DT, Sautin YY, Johnson RJ. Endogenous fructose production and metabolism in the liver contributes to the development of metabolic syndrome. *Nature Communications*. 2013; 4:2434.
43. Ingalls AM, Dickie MM, Snell GD. Obese, a new mutation in the house mouse. *Journal of Heredity*. 1950; 41(12):317–8. [PubMed: 14824537]
44. Klebanov S, Astle CM, DeSimone O, Ablamunits V, Harrison DE. Adipose tissue transplantation protects ob/ob mice from obesity, normalizes insulin sensitivity and restores fertility. *Journal of Endocrinology*. 2005; 186(1):203–11. [PubMed: 16002549]

45. Hummel KP, Dickie MM, Coleman DL. Diabetes, a new mutation in the mouse. *Science*. 1966; 153(3740):1127–8. [PubMed: 5918576]
46. Murano I, Barbatelli G, Parisani V, Latini C, Muzzonigro G, Castellucci M, Cinti S. Dead adipocytes, detected as crown-like structures, are prevalent in visceral fat depots of genetically obese mice. *Journal of Lipid Research*. 2008; 49(7):1562–8. [PubMed: 18390487]
47. Park YS, Uddin MJ, Piao L, Hwang I, Lee JH, Ha H. Novel role of endogenous catalase in macrophage polarization in adipose tissue. *Mediators Inflamm*. 2016; 2016:8675905. [PubMed: 27597806]
48. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *Journal of Clinical Investigation*. 2003; 112(12):1821–30. [PubMed: 14679177]
49. Kobayashi K, Forte TM, Taniguchi S, Ishida BY, Oka K, Chan L. The db/db mouse, a model for diabetic dyslipidemia: molecular characterization and effects of western diet feeding. *Metabolism*. 2000; 49(1):22–31. [PubMed: 10647060]
50. Baetens D, Stefan Y, Ravazzola M, Malaisse-Lagae F, Coleman DL, Orci L. Alteration of islet cell populations in spontaneously diabetic mice. *Diabetes*. 1978; 27(1):1–7. [PubMed: 340309]
51. Butler AE, Janson J, Soeller WC, Butler PC. Increased beta-cell apoptosis prevents adaptive increase in beta-cell mass in mouse model of type 2 diabetes: evidence for role of islet amyloid formation rather than direct action of amyloid. *Diabetes*. 2003; 52(9):2304–14. [PubMed: 12941770]
52. Goodpaster BH, Thaete FL, Kelley DE. Thigh adipose tissue distribution is associated with insulin resistance in obesity and in type 2 diabetes mellitus. *The American Journal of Clinical Nutrition*. 2000; 71(4):885–92. [PubMed: 10731493]
53. Goodpaster BH, Krishnaswami S, Resnick H, Kelley DE, Haggerty C, Harris TB, Schwartz AV, Kritchevsky S, Newman AB. Association between regional adipose tissue distribution and both type 2 diabetes and impaired glucose tolerance in elderly men and women. *Diabetes Care*. 2003; 26(2):372–9. [PubMed: 12547865]
54. Goth L, Eaton JW. Hereditary catalase deficiencies and increased risk of diabetes. *Lancet*. 2000; 356(9244):1820–1. [PubMed: 11117918]
55. Oliveira JM, Rebuffat SA, Gasa R, Gomis R. Targeting type 2 diabetes: lessons from a knockout model of insulin receptor substrate 2. *Canadian Journal of Physiology and Pharmacology*. 2014; 92(8):613–20. [PubMed: 24977713]
56. Terauchi Y, Matsui J, Suzuki R, Kubota N, Komeda K, Aizawa S, Eto K, Kimura S, Nagai R, Tobe K, Lienhard GE, Kadowaki T. Impact of genetic background and ablation of insulin receptor substrate (IRS)-3 on IRS-2 knock-out mice. *The Journal of Biological Chemistry*. 2003; 278(16):14284–90. [PubMed: 12493745]
57. O'Brien RM, Granner DK. Regulation of gene expression by insulin. *Physiological Reviews*. 1996; 76(4):1109–61. [PubMed: 8874496]
58. Gervois P, Fruchart JC, Staels B. Drug Insight: mechanisms of action and therapeutic applications for agonists of peroxisome proliferator-activated receptors. *Nature Clinical Practice Endocrinology and Metabolism*. 2007; 3(2):145–56.
59. Bermudez V, Finol F, Parra N, Parra M, Perez A, Penaranda L, Vilchez D, Rojas J, Arraiz N, Velasco M. PPAR-gamma agonists and their role in type 2 diabetes mellitus management. *Am J Ther*. 2010; 17(3):274–83. [PubMed: 20216208]
60. Lee YJ, Ko EH, Kim JE, Kim E, Lee H, Choi H, Yu JH, Kim HJ, Seong JK, Kim KS, Kim JW. Nuclear receptor PPARgamma-regulated monoacylglycerol O-acyltransferase 1 (MGAT1) expression is responsible for the lipid accumulation in diet-induced hepatic steatosis, PNAS. *Proceedings of the National Academy of Sciences USA*. 2012; 109(34):13656–61.
61. Matsusue K, Kusakabe T, Noguchi T, Takiguchi S, Suzuki T, Yamano S, Gonzalez FJ. Hepatic steatosis in leptin-deficient mice is promoted by the PPARgamma target gene Fsp27. *Cell Metabolism*. 2008; 7(4):302–11. [PubMed: 18396136]
62. Moran-Salvador E, Lopez-Parra M, Garcia-Alonso V, Titos E, Martinez-Clemente M, Gonzalez-Periz A, Lopez-Vicario C, Barak Y, Arroyo V, Claria J. Role for PPARgamma in obesity-induced

- hepatic steatosis as determined by hepatocyte- and macrophage-specific conditional knockouts. Federation of American Societies for Experimental Biology. 2011; 25(8):2538–50.
63. Frohnert BI, Sinaiko AR, Serrot FJ, Foncea RE, Moran A, Ikramuddin S, Choudry U, Bernlohr DA. Increased adipose protein carbonylation in human obesity. *Obesity Silver Spring*. 2011; 19(9): 1735–41. [PubMed: 21593812]
64. Elsner M, Gehrmann W, Lenzen S. Peroxisome-generated hydrogen peroxide as important mediator of lipotoxicity in insulin-producing cells. *Diabetes*. 2011; 60(1):200–8. [PubMed: 20971967]
65. Hoehn KL, Salmon AB, Hohnen-Behrens C, Turner N, Hoy AJ, Maghazal GJ, Stocker R, Van Remmen H, Kraegen EW, Cooney GJ, Richardson AR, James DE. Insulin resistance is a cellular antioxidant defense mechanism, PNAS. *Proceedings of the National Academy of Sciences U S A*. 2009; 106(42):17787–92.
66. Chen B, Wei J, Wang W, Cui G, Zhao Y, Zhu X, Zhu M, Guo W, Yu J. Identification of signaling pathways involved in aberrant production of adipokines in adipocytes undergoing oxidative stress. *Archives of Medical Research*. 2009; 40(4):241–8. [PubMed: 19608012]
67. Ruiz-Ojeda FJ, Gomez-Llorente C, Aguilera CM, Gil A, Ruperez AI. Impact of 3-Amino-1,2,4-Triazole (3-AT)-derived increase in hydrogen peroxide levels on inflammation and metabolism in human differentiated adipocytes. *PLoS One*. 2016; 11(3):e0152550. [PubMed: 27023799]
68. Grankvist K, Marklund SL, Taljedal IB. CuZn-superoxide dismutase, Mn-superoxide dismutase, catalase and glutathione peroxidase in pancreatic islets and other tissues in the mouse. *Biochemical Journal*. 1981; 199(2):393–8. [PubMed: 7041886]
69. Dedoussis GV, Kaliora AC, Panagiotakos DB. Genes, diet and type 2 diabetes mellitus: a review. *Rev Diabet Stud*. 2007; 4(1):13–24. [PubMed: 17565412]
70. Basciano H, Federico L, Adeli K. Fructose, insulin resistance, and metabolic dyslipidemia. *Nutr Metab (Lond)*. 2005; 2(1):5. [PubMed: 15723702]
71. Botezelli JD, Cambri LT, Ghezzi AC, Dalia RA, Voltarelli FA, de Mello MA. Fructose-rich diet leads to reduced aerobic capacity and to liver injury in rats. *Lipids Health Dis*. 2012; 11:78. [PubMed: 22713601]

Figure 1. Confirmation of *Cat*^{-/-} and changes in oxidative stress, body weight, and tissue weight in *Cat*^{-/-}

A. Liver homogenates from 12 mo old wild-type (WT) and catalase knockout (*Cat*^{-/-}) mice were probed with anti-catalase antibody. Representative immunoblots are shown. **B**. Oxidative stress in liver homogenates was estimated by the extent of protein carbonylation. Carbonylated proteins were identified using an anti-biotin antibody in Western blot analyses (upper figure) and subjected to densitometric analysis (lower figure). Data were normalized to untreated controls and expressed as a percentage of WT. Data are presented as the mean and associated SEM from 3 mice. * *P* < 0.05, Student's unpaired t-test, compared to WT. **C**. Body weight was measured in WT and *Cat*^{-/-} mice, fed a normal chow (6.5%) diet, over the period of 1.5-8 mo of age. Data are presented as the mean and associated SEM from 6 mice. * *P* < 0.05, Student's unpaired t test, compared to WT mice of same age. White adipose tissue (WAT) was harvested from 12 mo old *Cat*^{-/-} and WT mice and prepared for histological (paraffin embedded and stained with hematoxylin and eosin) analysis. **D**. Representative images of adipose tissue histology revealed hypertrophic and irregular adipocytes (noted by *) and crown structures (shown by an arrow).

Figure 2. Influence of catalase knockout on liver tissue

Liver was extracted from 12 mo old wild-type (WT) and catalase knockout (*Cat*^{-/-}) mice and prepared for histological (paraffin embedded and stained with hematoxylin and eosin) analysis. **A.** Liver histology. Lipid accumulations are identified by arrows (macrosteatosis) and circle (microsteatosis) (representative images shown). Histological orientation shown: portal triad (PT) and central vein (CV) 40x. **B.** Liver PPARγ expression was measured by immunoblot of liver homogenates (n=3) (representative blot shown; upper panel) and normalized to total protein (Ponceau stain) with densitometric analysis shown in density units (du) (lower panel). Data are expressed as mean ± SEM from 3 mice. * P < 0.05, Student's unpaired t-test, compared to WT.

Figure 3. Changes in glucose tolerance, fasting insulin, and pancreatic and muscle histology in catalase knockout mice

A A glucose tolerance test (GTT) was performed in 9 mo old wild-type (WT) and catalase knockout (*Car^{-/-}*) mice (n=3-5). **B**. The area under the curve (AUC) for the GTT was calculated for mice of both genotypes (n=3-5). Data are presented as mean and associated SEM. * $P < 0.05$, Student's unpaired t-test, compared to WT. Tissues were collected from 12-15 mo old mice, embedded in paraffin, sectioned and stained with hematoxylin and eosin. Images of pancreas (**C**) and gastrocnemius skeletal muscle (**E**) are shown at 40x magnification with insets at 200x magnification. Arrows indicate islets (**C**) or intermuscular adipose tissue (**E**). The areas of the islets (n=3) were determined using Image J software and are shown in (**D**). * $P < 0.05$, Student's unpaired t-test, compared to WT.

Table 1
RNA transcripts most altered in catalase knockout (*Cat*^{-/-}) mice

Genome-wide gene transcription profiling was obtained by RNA-sequencing of hepatic RNA obtained from two 2 mo old mice. RNA-seq was run on two samples from each genotype then, through biostatistical analysis. Data presented is combined from two animals. Genes with false discovery rate (FDR)-controlled $P < 0.05$ and fold-change (FC, >2 or <0.5) in *Cat*^{-/-} samples relative to WT samples were arbitrarily defined as being differentially-expressed. The transcripts in *Cat*^{-/-} mice that differed most from WT mice is ranked in order of statistical significance. Data were trimmed to include only known transcripts. The five most relevant genes that were up-regulated or down-regulated are listed.

Up-regulated genes				
Gene name	Gene	Log ₂ FC	FC	FDR
Cell Death-Inducing DFFA-Like Effector C	<i>Cidec</i>	6.949	124	2.1E-73
Transmembrane protein 28	<i>Tmem28</i>	6.889	119	3.0E-55
Oxysterol Binding Protein-Like 3	<i>Osbpl3</i>	5.053	33	1.6E-65
Complement factor D adipsin	<i>Cfd</i>	4.973	31	3.4E-12
Monoacylglycerol acyltransferase	<i>Mogat1</i>	4.715	26	5.6E-40
Down-regulated genes				
Gene name	Gene	log ₂ FC	FC	FDR
Catalase	<i>Cat</i>	-4.507	0.044	1.3E-96
Insulin-Like Growth Factor Binding Protein 1	<i>Igfbp1</i>	-4.493	0.044	1.7E-78
Protein phosphatase 1, regulatory (inhibitor) subunit 3G	<i>Ppp1r3g</i>	-3.947	0.065	4.7E-48
Chemokine (C-C motif) ligand 21A	<i>Ccl21a</i>	-3.153	0.11	8.8E-24
C-type lectin domain family 2, member h	<i>Clec2h</i>	-3.084	0.12	6.2E-24

Table 2
Pathophysiological changes in catalase knockout mice

Biochemical and morphological parameters were measured in tissues obtained from wild-type (WT) and catalase knockout (*Car*^{-/-}) mice. Liver and white adipose weights are expressed as the ratio of tissue weight-to-body weight (BW) as well as individually. Liver tissue was prepared for biochemical (triglyceride, alanine aminotransferase (ALT)) analyses. Data represent the mean and associated SEM from the number of mice indicated in parentheses.

Parameter	Age (mo) ^a	WT	<i>Car</i> ^{-/-}
Body weight (BW) (g)	12-15	38.2 ± 1.6 (9)	48.6 ± 2.3 * (9)
White adipose tissue weight (g)	12-15	2.7 ± 0.7 (3)	2.7 ± 0.4 (3)
Liver weight (g)	12-15	1.7 ± 0.1 (9)	2.6 ± 0.3 * (9)
White adipose tissue weight (% BW)	12-15	7.0 ± 1.5 (3)	6.1 ± 0.9 (3)
Liver weight (% BW)	12-15	4.3 ± 0.2 (9)	5.7 ± 0.4 * (9)
Serum triglycerides (mg/ml)	12	0.70 ± 0.03 (3)	0.86 ± 0.04 * (3)
Hepatic triglycerides (mM/g tissue)	12	5.3 ± 1.1 (3)	13.0 ± 2.0 * (3)
Alanine aminotransferase (ALT) (U/l)	12	114 ± 20 (3)	362 ± 116 (2)

^a Age of mice in which parameter was measured.

* P<0.05, Student's unpaired t-test, compared to WT.

Table 3
Liver damage associated with catalase knockout mice (shortened)

Male wild-type (WT) and catalase knockout (*Cat*^{-/-}) mice were sacrificed at >12mo of age. The liver was harvested, weighed, formalin fixed and then embedded in paraffin blocks and prepared for histological analysis using hematoxylin and eosin staining. Tissue damage was analyzed using the Brunt scoring method adapted for mice [41, 42]. Data represent the proportion of mice in each group meeting the pathological criterion.

Pathology	# mice showing pathology	
	WT	<i>Cat</i> ^{-/-}
<i>Macrosteatosis</i> ^a		
<10%	6/7	3/6
10-33%	1/7	3/6
>33%	0/6	1/6
<i>Microsteatosis</i> ^b		
any	1/7	5/6
<i>Inflammation</i> ^c		
<i>foci of inflammatory cells</i>	0/7	3/6
<i>lipogranulomas</i>	0/7	3/6
<i>foamy macrophages</i>	0/7	4/6
<i>Liver cell injury</i> ^d		
<i>ballooning degeneration</i>	0/7	2/6
<i>Fibrosis</i> ^e	0/7	0/6

^aVesicles larger than hepatocyte nucleus.

^bHepatocyte filled with small vesicles, central nucleus.

^cLooked for lobular inflammation, foci of inflammatory cells, lipogranulomas, portal inflammation, foamy macrophages.

^dLooked for ballooning, acidophil bodies, pigmented macrophages, megamitochondria.

^eDetermined by Picrosirius Red staining, imaging with polarized light in a tiling fashion across the whole tissue and analysis of the images by the 3I program Slidebook (3I, Denver, Colorado).

Table 4
Differentially-expressed liver transcripts in catalase knockout mice

Genes involved in metabolic dysfunction were identified through a literature search. Genome-wide gene transcription profiling was obtained by RNA-sequencing of hepatic RNA from 2 mo old mice (n=3). Genes with false discovery rate (FDR)-controlled $P < 0.05$ and fold-change (FC) > 2 or < 0.5 in *Cat*^{-/-} samples relative to WT samples were arbitrarily defined as being differentially-expressed.

Category	Gene	Gene symbol	Log ₂ FC	FC	FDR
Obesity	Leptin receptor	<i>Lepr</i>	-1.431	0.37	4.62E-08
	Beta ₂ adrenoceptors	<i>Adrb2</i>	-1.289	0.41	2.28E-03
Free fatty acid metabolism	alpha _{1A} adrenoceptors	<i>Adra1a</i>	1.657	3.2	6.36E-11
	Lipases	<i>Lipg</i>	1.251	2.4	1.25E-05
		<i>Lpl</i>	-1.749	0.30	3.88E-15
Insulin sensitivity	Peroxisome proliferator-activated receptor gamma (PPAR γ)	<i>Pparg</i>	2.273	4.8	1.66E-15
	Insulin receptor substrate	<i>Irs 2</i>	-1.804	0.29	7.81E-15
		<i>Irs 3</i>	1.633	3.1	2.90E-02
Lipid metabolism	Fatty acid translocase	<i>Cd36</i>	2.279	4.9	1.48E-18
	Apolipoprotein A 4	<i>Apoa4</i>	1.614	3.1	1.42E-09
Biosynthesis	3-Hydroxy-3-Methylglutaryl-CoA Reductase	<i>Hmgcr</i>	-1.122	0.46	1.77E-04
	Fatty Acid Synthase	<i>Fasn</i>	1.423	2.7	3.71E-12
	Stearoyl-CoA desaturase-1	<i>Scd1</i>	1.332	2.5	3.75E-10
	Stearoyl-CoA desaturase-2	<i>Scd2</i>	1.213	2.3	5.52E-07
	Stearoyl-CoA desaturase-3	<i>Scd3</i>	1.104	2.1	5.88E-07
	Stearoyl-CoA desaturase-4	<i>Scd4</i>	1.254	2.4	4.38E-09
Growth and differentiation	Ribosomal Protein S6 Kinase	<i>Rps6kal</i>	1.023	2.0	3.32E-06
Glucose transport	Solute Carrier Family 2 (Facilitated Glucose Transporter), Member 4 (glut4)	<i>Slc2a4</i>	2.128	4.4	2.40E-07

Table 5
Glucose and insulin changes in catalase knockout mice

Fasting blood glucose and serum insulin were measured in wild-type (WT) and catalase knockout (*Cat*^{-/-}) mice. Insulin levels were measured in 5 (before weight differences) and 8 (after weight differences) mo old WT and *Cat*^{-/-} mice. Data represent the mean and associated SEM from the number of mice indicated in parentheses.

Parameter	Age (mo) ^a	WT	<i>Cat</i> ^{-/-}
Fasting blood glucose (mg/dl)	12	157 ± 8 (3)	135 ± 16 (5)
Fasting serum insulin (pmol/l)	5	65.9 ± 0.3 (4)	67.1 ± 0.9 (4)
	8	69 ± 13 (3)	475 ± 158 [*] (3)

^a Age of mice in which parameter was measured.

^{*} P<0.05, Student's unpaired t-test, compared to WT.