Published in final edited form as:

Nat Med. 2009 November ; 15(11): 1307–1311. doi:10.1038/nm.2049.

Foxo1 integrates insulin signaling with mitochondrial function in the liver

Zhiyong Cheng¹, Shaodong Guo¹, Kyle Copps¹, Xiaochen Dong^{1,4}, Ramya Kollipara², Joseph T Rodgers³, Ronald A Depinho², Pere Puigserver³, and Morris F White¹

¹Howard Hughes Medical Institute, Division of Endocrinology, Children's Hospital Boston, Harvard Medical School, Boston, Massachusetts, USA

²Department of Medical Oncology, Belfer Institute for Applied Cancer Science, Departments of Medicine and Genetics, Harvard Medical School, Boston, Massachusetts, USA

³Department of Cancer Biology, Dana-Farber Cancer Institute, Department of Cell Biology, Harvard Medical School, Boston, Massachusetts, USA

Abstract

Type 2 diabetes is a complex disease that is marked by the dysfunction of glucose and lipid metabolism. Hepatic insulin resistance is especially pathogenic in type 2 diabetes, as it dysregulates fasting and postprandial glucose tolerance and promotes systemic dyslipidemia and nonalcoholic fatty liver disease^{1,2}. Mitochondrial dysfunction is closely associated with insulin resistance and might contribute to the progression of diabetes^{3,4}. Here we used previously generated mice⁵ with hepatic insulin resistance owing to the deletion of the genes encoding insulin receptor substrate-1 (Irs-1) and Irs-2 (referred to here as double-knockout (DKO) mice) to establish the molecular link between dysregulated insulin action and mitochondrial function. The expression of several forkhead box O1 (Foxo1) target genes increased in the DKO liver, including heme oxygenase-1 (Hmox1), which disrupts complex III and IV of the respiratory chain and lowers the NAD⁺/NADH ratio and ATP production. Although peroxisome proliferator-activated receptor- γ coactivator-1 α (Ppargc-1 α) was also upregulated in DKO liver, it was acetylated and failed to promote compensatory mitochondrial biogenesis or function. Deletion of hepatic *Foxo1* in DKO liver normalized the expression of Hmox1 and the NAD⁺/NADH ratio, reduced Ppargc-1 α acetylation and restored mitochondrial oxidative metabolism and biogenesis. Thus, Foxo1 integrates insulin signaling with mitochondrial function, and inhibition of Foxo1 can improve hepatic metabolism during insulin resistance and the metabolic syndrome.

© 2009 Nature America, Inc. All rights reserved.

Correspondence should be addressed to M.F.W. (morris.white@childrens.harvard.edu).

⁴Present address: Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana, USA.

AUTHOR CONTRIBUTIONS

Z.C. and M.F.W. designed this study. Z.C., S.G., K.C., X.D., R.K. and J.T.R. performed the experiments. Z.C. and M.F.W. analyzed the data. Z.C. and M.F.W. communicated with R.A.D. and P.P. about data interpretation and wrote the manuscript.

Reprints and permissions information is available online at <http://npg.nature.com/reprintsandpermissions/>.

Note: Supplementary information is available on the Nature Medicine website.

Irs1 and Irs2 are insulin receptor substrates, and their specific loss in the liver causes insulin resistance that progresses to hyperinsulinemia and diabetes^{5,6}. At 8 weeks of age, electron microscopy of liver sections from DKO mice revealed larger and lucent mitochondria that were distinctly different from the compact and electron-dense mitochondria in control liver (Fig. 1). The DKO liver contained twofold larger and 50% fewer mitochondria per hepatocyte than control liver (Fig. 1a,d,e), a substantial reduction that was confirmed by Mitotracker Green fluorescence and mitochondrial DNA (mtDNA) copy number (Supplementary Fig. 1). We detected similar mitochondrial abnormalities in 4-week-old DKO mice before hyperglycemia developed⁵, suggesting that insulin resistance rather than hyperglycemia dysregulated mitochondrial structure and biogenesis (Supplementary Fig. 2). Similar to DKO liver, liver sections from insulin-resistant leptin receptor-deficient (*db/db*) and leptin-deficient (*ob/ob*) mice revealed larger and fewer mitochondria than liver sections from control mice by electron microscopy (Fig. 1b,c,f,g). Therefore, direct (DKO mice) and indirect (*db/db* and *ob/ob* mice) genetic induction of insulin resistance was associated with dysregulated hepatic mitochondrial structure and biogenesis.

Foxo1 is a transcription factor that regulates genes controlling many hepatic functions, including glucose production and lipid metabolism^{5,7–10}. Under physiological conditions insulin inactivates Foxo1 by Akt-stimulated phosphorylation¹¹. Foxo1 was poorly phosphorylated in the liver of DKO, *db/db* and *ob/ob* mice (Supplementary Fig. 3). As a result, many hepatic genes were dysregulated in DKO liver, including several genes known to control mitochondrial function, biogenesis and dynamics (Supplementary Table 1). The concentrations of proteins that regulate mitochondrial fission and fusion—mitofusin-1, dynamin-1-like, fission-1 and dynactin-2—changed markedly in DKO, *db/db* and *ob/ob* mice, which was consistent with the accumulation of large and deformed mitochondria (Fig. 1h–j). As ablation of *Foxo1* ameliorates the metabolic syndrome in DKO mice (triple knockout (TKO) mice)⁵, we tested whether mitochondria were restored to normal in TKO mice. Indeed, expression of the dysregulated genes in TKO liver was restored to the normal range (Supplementary Table 1). Moreover, mitochondrial number and morphology and the expression of fusion and fission proteins were normalized in TKO mice and in *db/db* mice with *Foxo1* deletion (*db/db-Foxo1*^{−/−}, referred to as *db/db-FKO* mice hereafter) (Fig. 1a,b,d–i). Thus, Foxo1 inhibition, which normally occurs during insulin signaling, is required to achieve proper mitochondrial biogenesis and morphology in mouse liver during states of insulin resistance.

To investigate the effect of direct or indirect genetic induction of insulin resistance upon mitochondrial function, we examined several metabolic parameters in isolated mitochondria from DKO, *db/db* and *ob/ob* liver. Mitochondrial respiration couples O₂ consumption with ATP production, which is measured as the respiratory control ratio (RCR)—the ratio of the oxygen consumption rate in the presence of ADP (state 3) to that in the absence of ADP (state 4)^{12,13}. In the DKO, *db/db* and *ob/ob* mitochondria, state 3 respiration was substantially disrupted, but state 4 respiration was unchanged compared to control mitochondria (Supplementary Fig. 4a). Thus, the RCR was markedly lower in DKO, *db/db* and *ob/ob* mitochondria, showing that coupled mitochondrial respiration requires the Irs1 or Irs2 branch of the insulin signaling cascade (Fig. 2a). The mitochondrial ATP

production rate (APR) and cellular ATP concentration were also lower (Fig. 2b–c). Consistent with these results, the mitochondrial membrane potential decreased about 40% in DKO mitochondria (data not shown). Moreover, the electron transport chain (ETC) activity measured by NADH oxidation decreased 32% in DKO liver and about 50% in *db/db* and *ob/ob* liver compared to the controls (Fig. 2d). Consistent with the ETC deficiency, the NADH concentration increased and the NAD⁺ concentration decreased, which lowered the NAD⁺/NADH ratio in the DKO, *db/db* and *ob/ob* liver (Fig. 2e, Supplementary Fig. 4b). Mitochondrial dysfunction also developed in the liver of 4-week-old DKO mice that were insulin resistant but normoglycemic (Fig. 2f–h). Mitochondrial function was restored in TKO liver and *db/db*-FKO liver, as revealed by the normal RCR, APR, ATP concentration, ETC and NAD⁺/NADH ratio (Fig. 2a–e). Thus, activated Foxo1 is central in dysregulating mitochondrial function during hepatic insulin resistance.

Ppargc-1 α has a key role in mitochondrial biogenesis and function, as it induces the expression of nuclear respiration factor-1 (Nrf-1) and mitochondrial transcriptional factor A (Tfam)^{14,15}. The *Ppargc1a* level was 14-fold higher in DKO liver compared to the control liver (Supplementary Table 1), and the Ppargc-1 α concentration increased by two- to threefold in DKO liver and in *db/db* and *ob/ob* liver (Fig. 3a–c). However, we did not observe the expected increase in Nrf-1 and Tfam expression, which can explain, at least in part, the dysregulated liver mitochondrial function in these mice (Fig. 3a–c). To determine why Ppargc-1 α failed to promote mitochondrial biogenesis and function in the DKO liver, we measured the acetylation of Ppargc-1 α . Acetylation inhibits Ppargc-1 α function, and deacetylation is mediated by sirtuin-1 that is activated by NAD⁺ (ref. 16). Consistent with the low NAD⁺/NADH ratio in DKO, *db/db* and *ob/ob* liver, Ppargc-1 α was strongly acetylated (Fig. 3a–c). To corroborate that acetylation of Ppargc-1 α contributed to the dysregulation of DKO mitochondria, we infected DKO mice with an adenovirus encoding wild-type Ppargc-1 α or a mutant containing 13 lysine-to-arginine substitutions (R13-Ppargc-1 α) that prevent acetylation and thus inactivation of Ppargc-1 α ¹⁶. The concentration of total Ppargc-1 α increased substantially after adenoviral infection of wild-type and R13-Ppargc-1 α ; however, the abundance of acetylated Ppargc-1 α was markedly lower upon infection with R13-Ppargc-1 α (Fig. 3d). Moreover, mitochondrial biogenesis and morphology were acutely restored by R13-Ppargc-1 α but not by wild-type Ppargc-1 α , suggesting that acetylation contributed to mitochondrial dysregulation in DKO mice (Fig. 3e–g).

To determine whether Foxo1-induced mitochondrial dysfunction leads to the inhibition of compensatory Ppargc-1 α signaling, we investigated the integrity of the ETC. The ETC is composed of a series of sequentially acting electron carriers that consist of integral membrane proteins with prosthetic groups. Complexes I and II pass electrons from NADH and FADH₂ to complexes III and IV to generate the electrochemical proton gradient that drives ATP generation via complex V (ref 12). Complexes I, II and V, as indicated by the concentrations of NADH dehydrogenase subunit 6 (mt-Nd6), succinate dehydrogenase complex, subunit A (Sdha) and ATP synthase, H⁺ transporting, mitochondrial F1 complex, alpha subunit 1 (Atp5a1), respectively, were apparently unchanged in DKO, *db/db* and *ob/ob* mitochondria (Fig. 4a,b). By contrast, the essential components of the heme-

dependent complexes III and IV—ubiquinol-cytochrome c reductase core protein I (Uqcrc1) and cytochrome c oxidase subunit 1 (mt-Co1), respectively—were downregulated 200% in DKO, *db/db* and *ob/ob* mitochondria compared to controls (Fig. 4a,b).

Heme is an essential cofactor of mitochondrial oxidation, as it facilitates electron transport and ensures the expression, stability and function of complexes III and IV^{17–19}. Our observation of defects in heme-dependent (Uqcrc1 and mt-Co1) but not in heme-independent (mt-Nd6, *Sdha*, and *Atp5a1*) components implied an alteration of the mitochondrial heme pool in the insulin-resistant livers. Indeed, the mitochondrial heme concentration was 35% lower in DKO liver and about 50% lower in *db/db* and *ob/ob* liver (Fig. 4c). The reduction of heme content paralleled the upregulation of *Hmox1* in both gene and protein levels (Supplementary Table 1 and Fig. 4d). To determine whether the elevated expression of *Hmox1* could be responsible for the diminished heme concentration, we treated the primary hepatocytes from DKO, *db/db* or *ob/ob* mice with short interfering RNA (siRNA) targeted against *Hmox1* (Fig. 4e). The siRNA treatment lowered the concentration of *Hmox1* in DKO, *db/db* and *ob/ob* liver (Fig. 4e). Moreover, suppression of *Hmox1* largely normalized mitochondrial heme content in the isolated hepatocytes (Fig. 4f). Consistent with these results, siRNA against *Hmox1* restored ETC activity and the NAD⁺/NADH ratio to the normal range in DKO, *db/db* and *ob/ob* hepatocytes (Fig. 4g,h).

The deletion of hepatic *Foxo1* lowered *Hmox1* expression to the normal range and restored the expression of Uqcrc1 and mt-Co1 (Supplementary Table 1 and Fig. 4a–d). To establish whether *Hmox1* is a direct target of *Foxo1*, we prepared nuclear extracts from control, DKO and TKO livers for chromatin immunoprecipitation (ChIP) assays with antibody against *Foxo1* and PCR analysis of *Hmox1* promoter DNA. During insulin stimulation, the *Foxo1* concentration was 1.5-fold higher in DKO nuclear extracts compared to control extracts (consistent with the absence of insulin action) but entirely absent from TKO extracts (Fig. 4i). Accordingly, *Hmox1* promoter DNA abundance was twofold higher in nuclear extracts from DKO liver compared to controls and completely undetectable in extracts from TKO liver (Fig. 4i). Because the *Hmox1* promoter contains a *Foxo1* recognition motif (TATTTT), we conclude that *Foxo1* directly regulates *Hmox1*^{10,20}.

Our results reveal a complex network of genetic changes by which hepatic insulin resistance activates *Foxo1* and dysregulates mitochondrial biogenesis and function. *Foxo1* has a broad effect on hepatic gene expression, including *Hmox1* expression through a direct interaction with its promoter. *Hmox1* can dysregulate mitochondrial electron transport chain by decreasing the concentration of heme, which is required for the function and stability of complexes III and IV^{17–19}. Thus, sustained *Foxo1* activity decreased NADH oxidation, which decreases the concentration of NAD⁺ in the livers of DKO, *db/db* and *ob/ob* mice. Because a decreased NAD⁺/NADH ratio inactivates the NAD-dependent deacetylase sirtuin-1—which is expressed normally in DKO, *db/db* and *ob/ob* liver (Supplementary Table 1 and Supplementary Fig. 5)—Ppargc-1 α is strongly acetylated and unable to promote the mitochondrial biogenesis and function that are crucial for normal oxidative phosphorylation and hepatic nutrient homeostasis²¹. In line with this, resveratrol, which can compensate for reduced NAD concentration by lowering the Michaelis constant of sirtuin-1 for NAD⁺ (ref. 22), markedly decreased the abundance of acetylated Ppargc-1 α and restored

mitochondrial function and biogenesis in the primary hepatocytes from DKO and *ob/ob* mice (Supplementary Fig. 6). Foxo1 was also hyperacetylated in DKO liver (Supplementary Fig. 6); however, the effect of acetylation on Foxo1 activity remains controversial^{23,24}. Given that acetylation can stabilize and retain Foxo1 in the nucleus where it engages promyelocytic leukemia-associated protein (Pml) and undergoes activation^{23,24}, the redox inhibition of sirtuin-1 owing to mitochondrial dysfunction might augment Foxo1 activation and exacerbate its effects on metabolism in the insulin resistant liver. Thus, normalization of the NAD⁺/NADH ratio in DKO and *db/db* liver by deletion of *Foxo1*—or acute suppression of its target *Hmox1*—can indirectly reduce acetylation of Ppargc-1 α and restore normal mitochondrial number, morphology and function (Fig. 4j).

Foxo1 activation dysregulates several mitochondrial fusion and fission proteins in DKO, *db/db* and *ob/ob* mice, but whether Foxo1 has a direct effect on the expression of these genes is unknown. Regardless, deletion of Foxo1 normalizes the expression of these proteins and mitochondrial structure. The indirect inactivation of Ppargc-1 α by acetylation could account for the deformed mitochondria, as the expression of R13-Ppargc-1 α largely restored mitochondrial morphology and biogenesis in DKO liver. In skeletal muscle, Ppargc-1 α increases mitofusin protein expression by directly stimulating its gene expression²⁵.

One of the major complications in type 2 diabetes is the paradoxical accumulation of triglyceride and free fatty acids in the insulin-resistant liver²⁶. Considerable evidence shows that the maintenance of NAD⁺ concentration is required for normal mitochondrial β -oxidation^{27,28}. Pharmacological stimulation of mitochondrial NADH oxidation dramatically promotes fatty acid oxidation and ameliorates dyslipidemia, adiposity and fatty liver in obese mice²⁹. Consistent with the decreased NAD⁺ concentration, fatty acid oxidation was impaired and triglycerides accumulated in DKO liver (data not shown); however, deletion of Foxo1 from DKO liver normalized fatty acid oxidation and hepatic lipid content, concurrent with the restoration of mitochondrial function (data not shown). Thus, our results show how dysregulated insulin signaling can cause mitochondrial dysfunction that leads to metabolic disease. However, primary mitochondrial dysfunction can also promote fatty acid and triglyceride accumulation, which causes insulin resistance through the activation of serine kinases that inhibit Irs1 or Irs2 signaling³. In either case, partial inhibition of hepatic Foxo1 might be a good target to prevent the progression of a vicious cycle that causes metabolic disease.

ONLINE METHODS

Mice

We generated the liver-specific DKO and TKO mice as described previously⁵. We purchased *db/+* and *ob/+* breeding pairs (Jackson Laboratories) to produce *db/db* and *ob/ob* mice. We generated *db/db*-FKO mice by cross-breeding *db/+* mice with liver-specific *Foxo1*-knockout (FKO) mice⁵. We performed adenovirus infections via tail vein injection¹⁶. We housed all mice in plastic cages on a 12 h light-dark photocycle, giving them free access to water and food. We fasted the mice for 16 h and fed them with regular chow diet for 4 h before experiments. If not specified elsewhere, the control, DKO and TKO mice were 8–10 weeks old; the *db/+*, *db/db*, *db/db*-FKO, *ob/+* and *ob/ob* mice were 16–20 weeks old. We

performed mouse experiments according to procedures approved by the Children's Hospital Boston Institutional Animal Care and Use Committee.

Electron microscopy

We perfused the liver with 2% paraformaldehyde solution containing 2.5% glutaraldehyde, cut the perfused liver into small pieces ($1 \times 1 \times 2$ mm), fixed them in 5% glutaraldehyde at 4 °C overnight, postfixed them in 1% osmium tetroxide for 1 h at 25 °C, stained them in 2% uranyl acetate, dehydrated them in a series of ethanol dilutions (50–100%) and embedded them in epoxy resin (EMbed 812; Electron Microscopy Sciences). We stained the ultrathin sections (70 nm) with 2% uranyl acetate and lead citrate. We took five random sections from each liver and examined them in a Tecnai 12 BioTWIN electron microscope (FEI Co.), with ten random pictures taken from each section at various magnifications. We quantified mitochondrial number per cell was quantified by grid counting in a blinded fashion²¹. We measured mitochondrial area with Image 1.61 software (US National Institutes of Health)³⁰.

Measurement of enzyme activities

We measured ETC activity of mitochondrial suspensions as described³⁰, and mitochondrial ATP production rate with a luciferin-luciferase ATP monitoring reagent (Sigma-Aldrich)³¹. For the determination of steady-state ATP content in the liver, we prepared samples as reported previously³².

Oxygen consumption assays

We measured oxygen consumption with a Clark type polarographic oxygen probe (Micrometrix Corp.) as described previously^{13,33}. RCR was calculated as the ratios of the rate of oxygen uptake in state 3 to that in state 4.

Pyridine nucleotide measurements

We analyzed the pyridine nucleotides (NAD⁺ and NADH) in liver with EnzyChrom assay kits according to the manufacturer's instructions (BioAssay Systems).

Chromatin immunoprecipitation assay

We performed ChIP assay as described previously³⁴. Briefly, we minced mouse liver in cold PBS and passed the solution through a 21-gauge needle. We cross-linked the minced tissue with 1% formaldehyde in PBS for 15 min with constant shaking (25 °C). We performed the downstream assay with a ChIP assay kit according to the manufacturer's instructions (Upstate Biotechnology). We analyzed DNA by real-time PCR using an iQ SYBR Green Supermix (Bio-Rad). Forward and reverse primers for Hmox1 promoter were 5'-TGTGCCATCACTACCCAGAA-3' and 5'-AG CAGGGTAAGGCTTGGAAT-3' or 5'-TGGTTCCCAACTCACAGAGATCT-3' and 5'-GGGTAGTGATGGCACATTCCTT-3' (BioPolymers).

Immunoprecipitation and western blot

We performed immunoprecipitation and immunoblotting as described previously⁵. Protease inhibitor cocktail for lysate preparation was from Sigma, protein A/G agarose for

immunoprecipitation from Santa Cruz and nitrocellulose or polyvinylidene fluoride membranes from Amersham Pharmacia. We probed samples with antibodies and detected with horseradish peroxidase–conjugated secondary antibodies using ECL detection system (Perkin Elmer). The antibodies specific for Ppargc-1 α (sc-13067), NRF-1 (sc-33771 or ab34682), Tfam (sc-28200), mitofusin-1 (sc-50330), mitofusin-2 (sc-50331) and fission-1 (sc-48865) were from Santa Cruz or Abcam. Monoclonal antibodies specific for complex I (mt-Nd6), complex II (Sdha), complex III (Uqcrc1), complex IV (mt-Co1), and complex V (Atp5a1) were from Molecular Probes; antibodies to Foxo1 (9454S), Foxo1 phosphorylated on Thr24 (9464S) and β -actin (4970L) were from Cell Signaling Technology. Antibody to dynamin-1–like (NB110-55237) was from Novus Biologicals; dynactin-2 specific antibody (10031-2-AP) was from Proteintech Group; and lysine acetylation–specific antibody (05-515) was from Upstate. We analyzed and quantified images with Image Quant TL software (Amersham Biosciences). To focus on insulin effects after refeeding stimulation, we presented the data from refed mice only.

Statistical analyses

All results are expressed as means $\pm$ s.d. and are analyzed by analysis of variance to determine *P* values; *P* < 0.05 was considered statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank B. Spiegelman and L.M. Rohas for help with respiration assays, S. Orkin for help with fluorescent assays and R. de Cabo for the communication on flow cytometric assay. We are also grateful to Y. Long for his valuable comments and discussion. This project was supported by US National Institutes of Health grants DK38712 and DK55326 (M.F.W.), American Diabetes Association Mentor-Based Postdoctoral Fellowship 7-08-MN-63 (M.F.W. and Z.C.) and American Diabetes Association Junior Faculty Grant JF-7-07-27 (S.G.).

References

1. Edgerton DS, Johnson KM, Cherrington AD. Current strategies for the inhibition of hepatic glucose production in type 2 diabetes. *Front Biosci.* 2009; 14:1169–1181.
2. Postic C, Girard J. Contribution of *de novo* fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. *J Clin Invest.* 2008; 118:829–838. [PubMed: 18317565]
3. Lowell BB, Shulman GI. Mitochondrial dysfunction and type 2 diabetes. *Science.* 2005; 307:384–387. [PubMed: 15662004]
4. Kim JA, Wei Y, Sowers JR. Role of mitochondrial dysfunction in insulin resistance. *Circ Res.* 2008; 102:401–414. [PubMed: 18309108]
5. Dong XC, et al. Inactivation of hepatic Foxo1 by insulin signaling is required for adaptive nutrient homeostasis and endocrine growth regulation. *Cell Metab.* 2008; 8:65–76. [PubMed: 18590693]
6. Kubota N, et al. Dynamic functional relay between insulin receptor substrate 1 and 2 in hepatic insulin signaling during fasting and feeding. *Cell Metab.* 2008; 8:49–64. [PubMed: 18590692]
7. Matsumoto M, Poci A, Rossetti L, DePinho RA, Accili D. Impaired regulation of hepatic glucose production in mice lacking the forkhead transcription factor foxo1 in liver. *Cell Metab.* 2007; 6:208–216. [PubMed: 17767907]

8. Matsumoto M, Han S, Kitamura T, Accili D. Dual role of transcription factor FoxO1 in controlling hepatic insulin sensitivity and lipid metabolism. *J Clin Invest*. 2006; 116:2464–2472. [PubMed: 16906224]
9. Kamei Y, et al. Regulation of SREBP1c gene expression in skeletal muscle: role of retinoid X receptor/liver X receptor and forkhead-O1 transcription factor. *Endocrinology*. 2008; 149:2293–2305. [PubMed: 18202130]
10. Kamagate A, et al. FoxO1 mediates insulin-dependent regulation of hepatic VLDL production in mice. *J Clin Invest*. 2008; 118:2347–2364. [PubMed: 18497885]
11. van der Horst A, Burgering BM. Stressing the role of FoxO proteins in lifespan and disease. *Nat Rev Mol Cell Biol*. 2007; 8:440–450. [PubMed: 17522590]
12. Wallace DC. Mitochondrial diseases in man and mouse. *Science*. 1999; 283:1482–1488. [PubMed: 10066162]
13. Gottlieb E, Armour SM, Thompson CB. Mitochondrial respiratory control is lost during growth factor deprivation. *Proc Natl Acad Sci USA*. 2002; 99:12801–12806. [PubMed: 12228733]
14. Leone TC, et al. PGC-1 α deficiency causes multi-system energy metabolic derangements: muscle dysfunction, abnormal weight control and hepatic steatosis. *PLoS Biol*. 2005; 3:e101. [PubMed: 15760270]
15. Wu Z, et al. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell*. 1999; 98:115–124. [PubMed: 10412986]
16. Rodgers JT, Puigserver P. Fasting-dependent glucose and lipid metabolic response through hepatic sirtuin 1. *Proc Natl Acad Sci USA*. 2007; 104:12861–12866. [PubMed: 17646659]
17. Converso DP, et al. HO-1 is located in liver mitochondria and modulates mitochondrial heme content and metabolism. *FASEB J*. 2006; 20:1236–1238. [PubMed: 16672635]
18. Taillé C, et al. Induction of heme oxygenase-1 inhibits NAD(P)H oxidase activity by down-regulating cytochrome b558 expression via the reduction of heme availability. *J Biol Chem*. 2004; 279:28681–28688. [PubMed: 15123630]
19. González-Halphen D, Lindorfer MA, Capaldi RA. Subunit arrangement in beef heart complex III. *Biochemistry*. 1988; 27:7021–7031. [PubMed: 2848575]
20. Alam J, Cai J, Smith A. Isolation and characterization of the mouse heme oxygenase-1 gene. Distal 5' sequences are required for induction by heme or heavy metals. *J Biol Chem*. 1994; 269:1001–1009. [PubMed: 8288554]
21. Baur JA, et al. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature*. 2006; 444:337–342. [PubMed: 17086191]
22. Howitz KT, et al. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature*. 2003; 425:191–196. [PubMed: 12939617]
23. Liu Y, et al. A fasting inducible switch modulates gluconeogenesis via activator/ coactivator exchange. *Nature*. 2008; 456:269–273. [PubMed: 18849969]
24. Kitamura YI, et al. FoxO1 protects against pancreatic beta cell failure through NeuroD and MafA induction. *Cell Metab*. 2005; 2:153–163. [PubMed: 16154098]
25. Soriano FX, et al. Evidence for a mitochondrial regulatory pathway defined by peroxisome proliferator-activated receptor- γ coactivator-1 α , estrogen-related receptor- α , and mitofusin 2. *Diabetes*. 2006; 55:1783–1791. [PubMed: 16731843]
26. Brown MS, Goldstein JL. Selective versus total insulin resistance: a pathogenic paradox. *Cell Metab*. 2008; 7:95–96. [PubMed: 18249166]
27. Kunz WS. Evaluation of electron-transfer flavoprotein and α -lipoamide dehydrogenase redox states by two-channel fluorimetry and its application to the investigation of β -oxidation. *Biochim Biophys Acta*. 1988; 932:8–16. [PubMed: 3337800]
28. Lumeng L, Bremer J, Davis EJ. Suppression of the mitochondrial oxidation of (–)-palmitylcarnitine by the malate-aspartate and α -glycerophosphate shuttles. *J Biol Chem*. 1976; 251:277–284. [PubMed: 1245472]
29. Hwang JH, et al. Pharmacological stimulation of NADH oxidation ameliorates obesity and related phenotypes in mice. *Diabetes*. 2009; 58:965–974. [PubMed: 19136651]

30. Kelley DE, He J, Menshikova EV, Ritov VB. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes*. 2002; 51:2944–2950. [PubMed: 12351431]
31. Stump CS, Short KR, Bigelow ML, Schimke JM, Nair KS. Effect of insulin on human skeletal muscle mitochondrial ATP production, protein synthesis and mRNA transcripts. *Proc Natl Acad Sci USA*. 2003; 100:7996–8001. [PubMed: 12808136]
32. Manfredi G, Yang L, Gajewski CD, Mattiazzi M. Measurements of ATP in mammalian cells. *Methods*. 2002; 26:317–326. [PubMed: 12054922]
33. Peterside IE, Selak MA, Simmons RA. Impaired oxidative phosphorylation in hepatic mitochondria in growth-retarded rats. *Am J Physiol Endocrinol Metab*. 2003; 285:E1258–E1266. [PubMed: 14607783]
34. Friedman JR, et al. Orthogonal analysis of C/EBP β targets *in vivo* during liver proliferation. *Proc Natl Acad Sci USA*. 2004; 101:12986–12991. [PubMed: 15317935]

Figure 1.

Mitochondrial morphology and biogenesis in the insulin-resistant livers. (a–c) Electron microscopy study of mitochondrial biogenesis and morphology in the livers. Scale bar, 2 μ m. Cntr refers to the control (double floxed *Irs1* and *Irs2*) liver. (d,e) Mitochondrial number (d) and area (e) in control, DKO and TKO liver. (f,g) Mitochondrial number (f) and area (g) in control, *db/db*, *db/db-FKO* and *ob/ob* liver. (h–j) Top, immunoblotting detection of expression of mitochondrial fusion and fission proteins in control, DKO, TKO, *db/db*, *db/db-FKO* and *ob/ob* liver. Bottom, quantification of band density normalized to porin. Mfn1, mitofusin-1; Mfn2, mitofusin-2; Dnm1l, dynamin-1-like; Fis1, fission-1; Dctn2, dynactin-2. The results in bar graphs are presented as means $\pm$ s.d. ($n = 5$). * $P < 0.05$; ** $P < 0.01$.

Figure 2.

Evaluation of mitochondrial function. (a–e) RCR (a), APR (b), ATP content (c), ETC activity (d) and NAD⁺/NADH ratios (e) in the indicated groups of mice. The mice used in panels a–e were 8 weeks old, insulin resistant and hyperglycemic. (f–h) ETC activity (f), APR (g) and membrane potential (h) of the mitochondria from 4-week-old DKO mice that were insulin resistant but normoglycemic. All values are means $\pm$ s.d., $n = 4$; * $P < 0.05$; ** $P < 0.01$.

Figure 3.

Ppargc-1 α inactivation in liver. **(a)** Acetylation of Ppargc-1 α (acPpargc-1 α) and expression of downstream target proteins NRF-1 and Tfam in control, DKO and TKO liver, as determined by immunoprecipitation assay. **(b)** Acetylation of Ppargc-1 α and expression of downstream target proteins NRF-1 and Tfam in control, *db/db* and *db/db*-FKO liver, as determined by immunoprecipitation assay. **(c)** Acetylation of Ppargc-1 α and expression of downstream target proteins NRF-1 and Tfam in control and *ob/ob* liver, as determined by immunoprecipitation assay. **(d)** The expression of wild-type Ppargc-1 α , R13-Ppargc-1 α and the acetylation levels in DKO liver after adenovirus infection, as determined by immunoprecipitation assay. The bar graphs in panels **a–d** are density quantification of the related bands normalized to porin. **(e)** Electron microscopy assay of liver sections from DKO mice infected with adenoviruses expressing GFP, wild-type Ppargc-1 α and R13-Ppargc-1 α . Scale bar, 500 nm. **(f,g)** Mitochondrial (Mito) number **(f)** and area **(g)** in DKO liver infected with adenoviruses expressing GFP, wild-type Ppargc-1 α and R13-Ppargc-1 α . All values in bar graphs are means $\pm$ s.d., $n = 4$; * $P < 0.05$; ** $P < 0.01$.

Figure 4.

Foxo1 regulates electron transport chain via Hmox1. (a) The expression pattern of protein markers of complexes I–V (mt-Nd6, Sdhα, Uqcrc1, mt-Co1 and Atp5a1, respectively) in control, DKO and TKO liver. (b) The expression pattern of protein markers of complexes I–V (mt-Nd6, Sdhα, Uqcrc1, mt-Co1 and Atp5a1, respectively) in control, *db/db*, *db/db*-FKO and *ob/ob* liver. (c) Heme content measurement. (d) Expression profile of Hmox1 protein. (e) The effect of siRNA on Hmox1 expression. The bar graphs in panels a, b, d and e are density quantification of the related bands normalized to porin. (f–h) Effects of Hmox1 suppression on mitochondrial functions. White bar, primary hepatocytes from the control mice transfected with nonspecific siRNA (–); Gray bars, primary hepatocytes from DKO, *db/db* and *ob/ob* mice transfected with nonspecific siRNA (–) and Hmox1 siRNA (+), respectively. (i) ChIP assay of the molecular interaction between Foxo1 and *Hmox1* promoter. Left, immunoprecipitates subjected to immunoblot assay with Foxo1-specific antibody; whole tissue lysate was used as input control, and normal rabbit IgG was used as a negative control. Right, PCR and gel analysis using a pair of primers flanking the insulin response element (IRE) sequence in the *Hmox1* promoter. Double floxed Irs1 and Irs2 (dflox) and triple floxed Irs1, Irs2 and

Foxo1 (tflox) livers were used as the control of DKO and TKO livers, respectively. All values are the means $\pm$ s.d., $n = 4$; $*P < 0.05$; $**P < 0.01$. (j) A schematic model showing Foxo1 activation deregulates mitochondria under insulin resistance. Sirt1, sirtuin-1.