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The role of thiol redox switches in fatty liver disease

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Aims: Non-alcoholic fatty liver (NAFL) is a common liver disease associated with metabolic syndrome, obesity and diabetes that is rising in prevalence worldwide. Various molecular perturbations of key regulators and enzymes in hepatic lipid metabolism cause NAFL. However, redox regulation through glutathione (GSH) adducts in NAFL remains largely elusive. Glutaredoxin-1 (Glrx) is a small thioltransferase that removes protein GSH adducts without having direct antioxidant properties. The liver contains abundant Glrx but its metabolic function is unknown.

Results: Here we report that normal diet-fed Glrx-deficient mice ($Glrx^{-/-}$ spontaneously develop obesity, hyperlipidemia and hepatic steatosis by 8 months of age. Adenoviral Glrx repletion in the liver of $Glrx^{-/-}$ mice corrected lipid metabolism. $Glrx^{-/-}$ mice exhibited decreased sirtuin-1 activity that lead to hyper-acetylation and activation of SREBP-1 and upregulation of key hepatic enzymes involved in lipid synthesis. We found that GSH adducts inhibited SirT1 activity in $Glrx^{-/-}$ mice. Hepatic expression of non-oxidizable

cysteine mutant SirT1 corrected hepatic lipids in $Glrx^{-/-}$ mice. Wild type mice fed high fat diet develop metabolic syndrome, diabetes and NAFL within several months. Glrx-deficiency accelerated high fat-induced NAFL and progression to steatohepatitis, manifested by hepatic damage and inflammation.

Innovation: These data suggest an essential role of hepatic Glrx, which controls protein glutathione adducts in the pathogenesis of hepatic steatosis.

Conclusion: We provide a novel redox-dependent mechanism for regulation of hepatic lipid metabolism, and propose that upregulation of hepatic Glrx may be a beneficial strategy for NAFL.

Short Biography

Markus Bachschmid

NAME: Markus Bachschmid
POSITION TITLE: Assistant Professor of Medicine

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date MM/YYYY	FIELD OF STUDY
Marianum, Buxheim	Baccalaureate	1992	Biology, Mathematics, Geography, German
University of Konstanz	B. Sc.	1995	Biology
University of Konstanz	M. Sc.	1998	Biology
University of Konstanz	Ph. D.	2003	Biochemistry, Pharmacology, Toxicology

A. Personal Statement

Dr. Bachschmid is an Assistant Professor of Medicine at the Boston University School of Medicine (BUSM) and a Faculty Member of the Whitaker Cardiovascular Institute, Graduate Program in Molecular and Translational Medicine and Graduate Medical Sciences. Dr. Bachschmid has authored/coauthored more than 70 peer-review publications, reviews and textbook chapters. He has served as an expert reviewer for various national and international organizations including the American Heart Association, COMET (Austria), NASA, NIH, Research Grants Council of Hong Kong (China) and Israel Science Foundation (Israel) and numerous international journals including Antioxidants & Redox Signaling, The FASEB Journal, JPET, American Journal of Physiology, Circulation, and Atherosclerosis. Dr. Bachschmid also serves as an Academic Editor for the largest international open access journal PLOS ONE published by the Public Library of Science. He recently launched as a co-director the "Metabolic Clinical Research Collaborative" at Boston University School of Medicine (Affinity Research Collaborative-ARC), which promotes translational research of both, basic and clinical research scientists.

The main focus of Dr. Bachschmid's laboratory have been on redox regulation and post-translational protein modifications including cysteine oxidation and lysine acetylation affecting cellular signaling cascades and the metabolic master regulator Sirtuin-1, which is funded by the NIH and AHA.

Dr. Bachschmid's laboratory uses comprehensive multidisciplinary approaches to study fundamental biological redox processes ranging from chemistry to animal models and human pathology. Various methodologies are established in his laboratory, including a wide variety of molecular and biochemical assays, systems biology, metabolism, *in vivo* viral transfection strategies using adeno and adeno-associated viruses, and a novel isotope coded multiplex proteomics platform to identify and measure global protein modifications in tissue and body fluid samples.

A publication on metabolic effects of glutaredoxin-1 deficiency is under revision in "Antioxidants & Redox Signaling", which is the most prestigious journal in the field of oxidants and redox regulation (see below).

1. Yao C, Behring JB, Shao D, Sverdlov AL, Whelan SA, Elezaby A, Yin X, Siwik DA, Seta F, Costello CE, Cohen RA, Matsui R, Colucci WS, McComb ME, **Bachschmid MM**. Overexpression of Catalase Diminishes Oxidative Cysteine Modifications of Cardiac Proteins. PLoS One. 2015;10(12):e0144025. PMID: 26642319
2. Behring JB, Kumar V, Whelan SA, Chauhan P, Siwik D, Costello CE, Colucci WS, Cohen RA, McComb ME, **Bachschmid MM**. Does reversible cysteine oxidation link the Western Diet to cardiac dysfunction? FASEB J. 28(5):1975-87, 2014. PMID: 24469991
3. Shao D, Han J, Hou X, Fry J, Behring JB, Seta F, Long MT, Roy HK, Cohen RA, Matsui R, **Bachschmid MM.** Glutaredoxin-1 deficiency causes fatty liver and dyslipidemia by inhibiting sirtuin-1. Antiox Redox Signaling 2016. Epub ahead of print.

B. Positions and Honors

2012-present	Assistant Professor of Medicine, Vascular Biology Section, Boston University
2007-2012	Research Assistant Professor of Medicine, Vascular Biology Section, Boston University
2006-2007	Research Associate, Vascular Biology Unit, Boston University School of Medicine, Boston, MA
2003-2006	Post Doc in the laboratory of Dr. V. Ullrich, Biological Chemistry, Department of Biology, University of Konstanz, Germany
2001-2005	Member and scholarship holder of the "Graduiertenkolleg" for Biomedical Drug Research supported by Altana Pharma, German Research Foundation (DFG) and the University of Konstanz.
1999-2001	Member and scholarship holder of the "Graduiertenkolleg" for Biochemical Pharmacology supported by German Research Foundation (DFG) and the University of Konstanz.
2004	Altana Pharma Award, Konstanz, Germany
2003	Summa Cum Laude, University of Konstanz, Konstanz, Baden-Wuerttemberg, Germany
2003	Poster award for the best poster at 1st joint French-German •NO meeting
1992	"Jugend Forscht" Special Award for Environment: „Effects of Heavy Metal Salts on Spinach"
2010-present	American Heart Association's Vascular Wall Bio BSc1 Peer Review Study Group

C. Contribution to Science

1. Endothelial Dysfunction

Endothelial dysfunction is clinically diagnosed by angiography following injection of acetylcholine into the coronary vessel. Acetylcholine causes nitric oxide-dependent vasorelaxation in a healthy artery; however, administered to a blood vessel with dysfunctional endothelium, acetylcholine causes "paradoxical" vasoconstriction and vasospasm. During my time as a PhD student, I was working with my mentor Dr. M. H. Zou, now the Founding Director of Center for Molecular and Translational Medicine at Georgia State University, on the molecular mechanism explaining endothelial dysfunction, impaired vasorelaxation and vasospasm. We reproduced the vasospasm in isolated bovine coronary arteries by promoting inflammation or oxidative stress (ref. 1,2). We found that the vasospasm was caused by an imbalance of various vasoactive mediators involving nitric

oxide, superoxide, prostacyclin and prostaglandin endoperoxide H_2. In the dysfunctional endothelium, the radical superoxide increases which inactivates nitric oxide, to form the highly reactive molecule peroxynitrite. Thus the increase in superoxide limits nitric oxide bioavailability to mediate relaxation. The newly formed peroxynitrite inactivates prostacyclin synthase that produces prostacyclin for vasorelaxation. As a consequence, the substrate of prostacyclin synthase, PGH_2, increases. PGH_2 has potent vasconstrictive properties, similar to thromboxane, promoting vasospasm. This process also occurs in inflammation and is part of the innate immune response leading to endothelial cell activation (figure). Endothelial cell activation is required to change the cell surface properties and hemodynamics, allowing immune cell invasion. Chronic inflammation, however, will maintain this properties leading to endothelial dysfunction. Chronic inflammation, as determined by C-reactive protein, is now a recognized additional risk factor for cardiovascular disease. During my time as a PhD student, together with Dr. T. Luescher (University of Zurich, Switzerland), I showed that superoxide-diminished NO bioavailability is a hallmark of endothelial dysfunction (ref. 3,4).

For my thesis about the molecular mechanism of superoxide and prostacyclin nitration regulating blood vessel tone, I was awarded with the Altana Pharma Prize, a nationally recognized award (http://www.aerzteblatt.de/archiv/42829/Verleihungen in German).

1. Zou, MH, **Bachschmid M.** Hypoxia-reoxygenation triggers coronary vasospasm in isolated bovine coronary arteries via tyrosine nitration of prostacyclin synthase. J. Exp. Med. 1999;190:135-139. PMID: 10429677
2. **Bachschmid M**, Thurau S, Zou MH, Ullrich V. Endothelial cell activation by endotoxin involves superoxide/NO-mediated nitration of prostacyclin synthase and thromboxane receptor stimulation. FASEB J. 2003;17:914-916. PMID: 12670882
3. van der Loo B, Labugger R, Skepper JN, **Bachschmid M**, Kilo J, Powell JM, Palacios-Callender M, Erusalimsky JD, Quaschning T, Malinski T, Gygi D, Ullrich V, Luscher TF. Enhanced peroxynitrite formation is associated with vascular aging. J Exp Med. 2000;192:1731-1744. PMID: 11120770
4. Cosentino F, Eto M, De Paolis P, van der Loo B, **Bachschmid M**, Ullrich V, Kouroedov A, Delli Gatti C, Joch H, Volpe M, Luscher TF. High glucose causes upregulation of cyclooxygenase-2 and alters prostanoid profile in human endothelial cells: role of protein kinase C and reactive oxygen species. Circulation. 2003;107:1017-1023. PMID: 12600916

2. Regulation of Cyclooxygenase Activity

Cyclooxygenase (COX) activity is important for vascular homeostasis, platelet aggregation and inflammation. COX is a complex enzyme comprised of two catalytically distinct domains; peroxidase and cyclooxygenase domain. Oxidant species can activate the enzyme by acting as a cosubstrate for the peroxidase domain. This primes the COX domaine to utilize arachidonic acid and produce the endoperoxide H_2 (PGH_2). I have shown that in platelets, this can lead to hyper-activation promoting thrombus formation and occlusive vascular disease (ref. 1).

Acetaminophen (APAP, Tylenol), through its phenolic structure, is a potent radical scavenger. I have proposed a new mechanism for the inhibition of COX, by which Tylenol inactivates the co-substrate peroxynitrite leading to inhibition of the cyclooxygenase (ref. 2).

Furthermore, I have shown that COX-2 isoform specific inhibitors may have adverse effects by inhibiting the synthesis of vasoprotective prostacyclin in the vascular smooth

muscle layer (media) of blood vessels (ref. 3). These findings may explain the increased risk of cardiovascular complications associated with COX-2 specific inhibitors such as Vioxx.

Furthermore, I have investigated a mechanism by which endogenous nitrite in inflamed tissues may serve as an endogenous inhibitor of COX-2 to limit inflammation (ref. 4). The enzyme can convert nitrite to a reactive intermediate that blocks the active site tyrosine in its COX domain. This finding was a featured article in "Antioxidant and Redox Signaling", the most reputed journal for redox biology. Nitrite is now recognized as a beneficial bioactive molecule in medicine.

1. Schildknecht S, van der Loo B, Weber K, Tiefenthaler K, Daiber A, **Bachschmid MM**. Endogenous peroxynitrite modulates PGHS-1-dependent thromboxane A2 formation and aggregation in human platelets. Free Radic Biol Med. 2008;45:512-520. PMID: 18514074
2. Schildknecht, S, Daiber, A, Ghisla, S, Cohen, RA, **Bachschmid, MM**. Acetaminophen inhibits prostanoid synthesis by scavenging the PGHS-activator peroxynitrite. FASEB J. 2008;22(1):215-224. PMID: 17724253
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3. Metabolic Cardiovascular and Liver Disease

After completing my post-doctoral training in Germany and at Boston University, I was promoted to Research Assistant Professor under Dr. R. A. Cohen. As faculty, I began studying the role of protein cysteine oxidation of Ras and Sirtuin-1 in metabolic cardiovascular disease.

Firstly, my laboratory demonstrated that metabolic stress-induced cysteine oxidation of Ras (in vitro and in mice) sequesters Ras in the golgi apparatus and prevents growth factor receptor signaling (ref. 1). This novel mechanism could explain growth factor resistance in diabetic patients (VIVA, FIRST and AGENT clinical trials) resulting in impaired angiogenesis.

Secondly, studies on the important metabolic regulator Sirtuin-1, showed that oxidants can inhibit its activity (ref. 2,3) and interfere with protein-protein interactions. My laboratory identified three critical oxidant sensitive cysteines, one of which was later confirmed to play a critical role in angiogenesis (PMCID: PMC3864331). My laboratory has created a triple cysteine to serine mutant that is oxidant resistant and protects hepatocytes from metabolic and oxidative stress-induced apoptosis (ref. 3).

In a recent effort to identify additional proteins with altered cysteine oxidation caused metabolic stress, my laboratory has established a novel proteomics method with isotope coded tandem mass tags, allowing identification and quantification of reversibly oxidized cysteine residues. This screen in the left ventricle of a mouse model for metabolic syndrome and diastolic dysfunction revealed perturbations of mitochondrial electron transport chain components (bottom figure, ref. 4). In collaboration with Dr. W. S. Colucci, we validated the changes in reversible oxidation of complex II and defined their

role as major contributor to mitochondrial oxidant generation and inhibition of ATP synthesis.

1. Burgoyne JR, Haeussler, DJ, Kumar V, Ji Y, Pimental DR, Zee RS, Costello CE, Lin C, McComb ME, Cohen RA, **Bachschmid MM**. Oxidation of HRas cysteine thiols by metabolic stress prevents palmitoylation in vivo and contributes to endothelial cell apoptosis. FASEB J. 2012;26:832-841. PMID: 22085642
2. Zee RS, Yoo CB, Pimentel DR, Perlman DH, Burgoyne JR, Hou X, McComb ME, Costello CE, Cohen RA, **Bachschmid MM**. Redox regulation of sirtuin-1 by S-glutathiolation. Antioxid Redox Signal. 2010;13:1023-1032. PMID: 20392170
3. Shao D, Fry JL, Han J, Hou X, Pimentel DR, Matsui R, Cohen RA, **Bachschmid MM**. A redox-resistant sirtuin-1 mutant protects against hepatic metabolic and oxidant stress. J Biol Chem. 2014;289:7293-7306. PMID: 24451382
4. Behring JB, Kumar V, Whelan SA, Chauhan P, Siwik DA, Costello CE, Colucci WS, Cohen RA, McComb ME, **Bachschmid MM**. Does reversible cysteine oxidation link the Western diet to cardiac dysfunction? FASEB J. 2014;28:1975-1987. PMID: 24469991

D. Research Support

Active

DK103750 (PI Bachschmid) 07/01/15-06/30/2020
NIDDK
Redox control of hepatic lipid metabolism.
The major goal of this project is to identify molecular mechanisms that cause non-alcoholic fatty liver disease in glutaredoxin-1 ablated mice.

16GRNT27660006 (PI Bachschmid) 01/01/16-12/31/2018
AHA
Impaired cysteine-thiol redox signaling in metabolic cardiovascular disease
The major goal of this grant is to identify novel protein target in the heart that are regulated by reversible cysteine oxidation and become dysregulated in metabolic cardiovascular disease.

9500305618 (PI Bachschmid) 4/1/2016-3/31/2017
CTSI
Sirtuin-1 glutathione adducts as a target to treat non-alcoholic fatty liver disease
The major goal of this grant is to identify gene expression changes in a Glutaredoxin-1 KO mouse model that are related to oxidative stress and SirT1-regulated activity of transcription factors.

HL1195955 (PI London, Subcontract Bachschmid) 08/15/13–5/31/17
NSBRI
Regulation of the Cardiac Sodium Channel by SIRTUIN1.
The major goals of this project are The major goals of this project is to identify with mass spectrometry Sirtuin-1 dependent acetylation sites on the cardiac sodium channel SCN5a.

R01HL064750 (PI Colucci; Subcontract Bachschmid) 7/01/2015-06/30/2020
NIH/NHLBI
Oxidative stress in myocardial remodeling and failure.
The major goals of this project are to define the role of oxidative stress on the endoplasmic reticulum and the associated cross communication with mitochondria.

Complete list (total of 77) of published work in ORCID (0000-0002-0748-552) or NCBI

1. Kumar S, Kim YR, Vikram A, Naqvi A, Li Q, Kassan M, Kumar V, Bachschmid MM, Jacobs JS, Kumar A, Irani K. Sirtuin1-regulated lysine acetylation of p66Shc governs diabetes-induced vascular oxidative stress and endothelial dysfunction. Proceedings of the National Academy of Sciences of the United States of America. 2017; 114(7):1714-1719. PubMed [journal] PMID: 28137876, PMCID: PMC5321021

2. Vikram A, Lewarchik CM, Yoon JY, Naqvi A, Kumar S, Morgan GM, Jacobs JS, Li Q, Kim YR, Kassan M, Liu J, Gabani M, Kumar A, Mehdi H, Zhu X, Guan X, Kutschke W, Zhang X, Boudreau RL, Dai S, Matasic DS, Jung SB, Margulies KB, Kumar V, Bachschmid MM, London B, Irani K. Sirtuin 1 regulates cardiac electrical activity by deacetylating the cardiac sodium channel. Nature medicine. 2017; PubMed [journal] PMID: 28191886

3. Gorelenkova Miller O, Behring JB, Siedlak SL, Jiang S, Matsui R, Bachschmid MM, Zhu X, Mieyal JJ. Upregulation of Glutaredoxin-1 Activates Microglia and Promotes Neurodegeneration: Implications for Parkinson's Disease. Antioxidants & redox signaling. 2016; 25(18):967-982. PubMed [journal] PMID: 27224303, PMCID: PMC5175443

4. Shao D, Han J, Hou X, Fry JL, Behring JB, Seta F, Long M, Roy H, Cohen RA, Matsui R, Bachschmid MM. Glutaredoxin-1 deficiency causes fatty liver and dyslipidemia by inhibiting sirtuin-1. Antioxidants & redox signaling. 2016; PubMed [journal] PMID: 27958883

5. Han J, Weisbrod RM, Shao D, Watanabe Y, Yin X, Bachschmid MM, Seta F, Janssen-Heininger YM, Matsui R, Zang M, Hamburg NM, Cohen RA. The redox mechanism for vascular barrier dysfunction associated with metabolic disorders: Glutathionylation of Rac1 in endothelial cells. Redox biology. 2016; 9:306-319. PubMed [journal] PMID: 27693992, PMCID: PMC5045950

6. Fry JL, Al Sayah L, Weisbrod RM, Van Roy I, Weng X, Cohen RA, Bachschmid MM, Seta F. Vascular Smooth Muscle Sirtuin-1 Protects Against Diet-Induced Aortic Stiffness. Hypertension (Dallas, Tex. : 1979). 2016; 68(3):775-84. NIHMSID: NIHMS795301 PubMed [journal] PMID: 27432859, PMCID: PMC4982825

7. Cohen RA, Murdoch CE, Watanabe Y, Bolotina VM, Evangelista AM, Haeussler DJ, Smith MD, Mei Y, Tong X, Han J, Behring JB, Bachschmid MM, Matsui R. Endothelial Cell Redox Regulation of Ischemic Angiogenesis. Journal of cardiovascular pharmacology. 2016; 67(6):458-64. NIHMSID: NIHMS761325 PubMed [journal] PMID: 26927696, PMCID: PMC4899292

8. Watanabe Y, Murdoch CE, Sano S, Ido Y, Bachschmid MM, Cohen RA, Matsui R. Glutathione adducts induced by ischemia and deletion of glutaredoxin-1 stabilize HIF-1α and improve limb revascularization. Proceedings of the National Academy of Sciences of the United States of America. 2016; 113(21):6011-6. PubMed [journal] PMID: 27162359, PMCID: PMC4889374

9. Ji Y, Bachschmid MM, Costello CE, Lin C. S- to N-Palmitoyl Transfer During Proteomic Sample Preparation. Journal of the American Society for Mass Spectrometry. 2016; 27(4):677-85. NIHMSID: NIHMS749183 PubMed [journal] PMID: 26729453, PMCID: PMC4794353

10. Peskin AV, Pace PE, Behring JB, Paton LN, Soethoudt M, Bachschmid MM, Winterbourn CC. Glutathionylation of the Active Site Cysteines of Peroxiredoxin 2 and Recycling by

Glutaredoxin. The Journal of biological chemistry. 2016; 291(6):3053-62. PubMed [journal] PMID: 26601956, PMCID: PMC4742766

11. Sverdlov AL, Elezaby A, Qin F, Behring JB, Luptak I, Calamaras TD, Siwik DA, Miller EJ, Liesa M, Shirihai OS, Pimentel DR, Cohen RA, Bachschmid MM, Colucci WS. Mitochondrial Reactive Oxygen Species Mediate Cardiac Structural, Functional, and Mitochondrial Consequences of Diet-Induced Metabolic Heart Disease. Journal of the American Heart Association. 2016; 5(1). PubMed [journal] PMID: 26755553, PMCID: PMC4859372

12. Miller EJ, Calamaras T, Elezaby A, Sverdlov A, Qin F, Luptak I, Wang K, Sun X, Vijay A, Croteau D, Bachschmid M, Cohen RA, Walsh K, Colucci WS. Partial Liver Kinase B1 (LKB1) Deficiency Promotes Diastolic Dysfunction, De Novo Systolic Dysfunction, Apoptosis, and Mitochondrial Dysfunction With Dietary Metabolic Challenge. Journal of the American Heart Association. 2015; 5(1). PubMed [journal] PMID: 26722122, PMCID: PMC4859355

13. Yao C, Behring JB, Shao D, Sverdlov AL, Whelan SA, Elezaby A, Yin X, Siwik DA, Seta F, Costello CE, Cohen RA, Matsui R, Colucci WS, McComb ME, Bachschmid MM. Overexpression of Catalase Diminishes Oxidative Cysteine Modifications of Cardiac Proteins. PloS one. 2015; 10(12):e0144025. PubMed [journal] PMID: 26642319, PMCID: PMC4671598

14. Fry JL, Shiraishi Y, Turcotte R, Yu X, Gao YZ, Akiki R, Bachschmid M, Zhang Y, Morgan KG, Cohen RA, Seta F. Vascular Smooth Muscle Sirtuin-1 Protects Against Aortic Dissection During Angiotensin II-Induced Hypertension. Journal of the American Heart Association. 2015; 4(9):e002384. PubMed [journal] PMID: 26376991, PMCID: PMC4599512

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20. Shao D, Fry JL, Han J, Hou X, Pimentel DR, Matsui R, Cohen RA, Bachschmid MM. A redox-resistant sirtuin-1 mutant protects against hepatic metabolic and oxidant stress. The Journal of biological chemistry. 2014; 289(11):7293-306. PubMed [journal] PMID: 24451382, PMCID: PMC3953247

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