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

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE</th>
<th>Completion Date</th>
<th>FIELD OF STUDY</th>
</tr>
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<tbody>
<tr>
<td>Marianum, Buxheim</td>
<td>Baccalaureate</td>
<td>1992</td>
<td>Biology, Mathematics, Geography, German</td>
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<tr>
<td>University of Konstanz</td>
<td>B. Sc.</td>
<td>1995</td>
<td>Biology</td>
</tr>
<tr>
<td>University of Konstanz</td>
<td>M. Sc.</td>
<td>1998</td>
<td>Biology</td>
</tr>
<tr>
<td>University of Konstanz</td>
<td>Ph. D.</td>
<td>2003</td>
<td>Biochemistry, Pharmacology, Toxicology</td>
</tr>
</tbody>
</table>

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).


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 vasoconstrictive 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).


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


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 domain 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.


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.


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


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


30. Lugus JJ, Ngoh GA, Bachschmid MM, Walsh K. Mitofusins are required for angiogenic function and modulate different signaling pathways in cultured endothelial cells. Journal


73. Ullrich V, Zou MH, Bachschmid M. New physiological and pathophysiological aspects


