formation, which forms free radicals and exacerbates inflammation in $CCl_4$-induced early steatohepatitic lesions in obese mice. This is a first report of the role of leptin in augmenting macrophage dependent, peroxynitrite-induced, free radical-mediated tissue damage in steatohepatitis of obesity.

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## Ethanol and Reactive Species Increase Basal Sequence Heterogeneity of Hepatitis C Virus and Produces Variants With Reduced Susceptibility To Antivirals

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Hepatitis C virus exhibits a high level of genetic variability, and variants with reduced susceptibility to antivirals can occur even before treatment begins. in addition, alcohol decreases efficacy of antiviral therapy and increases sequence heterogeneity of HCV RNA but how ethanol affects HCV sequence is unknown. Ethanol metabolism and HCV infection increase the level of reactive species that can alter cell metabolism and signaling, and potentially act as mutagen to the viral RNA. Therefore, we investigated whether ethanol and reactive species affected the basal sequence heterogeneity of HCV RNA in hepatocytes. Human hepatoma cells supporting a continuous replication of genotype 1b HCV RNA were exposed to ethanol, acetaldehyde, hydrogen peroxide, or L-buthionine-S,R-sulfoximine (BSO) that decreased intracellular glutathione as seen in patients. Then, NS5A region was sequenced and compared with genotype 1b HCV sequences in the HCV database. Ethanol and BSO elevated nucleotide and amino acid substitution rates of hepatitis C virus RNA by 4–18 folds within 48 hrs which were accompanied by oxidative RNA damage. Iron chelator and glutathione ester decreased both RNA damage and mutation rates. Furthermore, infectious HCV and HCV core gene were sufficient to induce oxidative RNA damage even in the absence of ethanol or BSO. Interestingly, the dn/ds ratio and percentage of sites undergoing positive selection increased with ethanol and BSO, resulting in an increased detection of NS5A variants with reduced susceptibility to interferon alpha, cyclosporine, and ribavirin and others implicated in immune tolerance and modulation of viral replication. Tyrosine phosphorylation of Stat1 increased with ethanol and BSO treatments, suggesting a role of redox signaling. Therefore, virus-induced oxidative/nitrosative stress is likely to synergize with ethanol to modulate the basal mutation rate of HCV and contribute to antiviral resistance through positive selection.

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## Myeloperoxidase-derived Oxidants Inhibit SERCA Activity and Perturb $Ca^{2+}$ Homeostasis in Human Coronary Artery Endothelial Cells

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The sarco/endoplasmic reticulum $Ca^{2+}$-ATPase (SERCA) plays a critical role in regulating intracellular $Ca^{2+}$ homeostasis by sequestering cytosolic $Ca^{2+}$. Several oxidants generated by activated immune cells at sites of inflammation have been shown to modulate SERCA activity, and is established that the activity of this pump is impaired in ageing tissues and cardiovascular disease. We have shown previously that hypothiocyanous acid (HOSCN), which is generated at high levels in smokers by the heme enzyme, myeloperoxidase (MPO), induces cellular dysfunction, but the mechanism(s) involved are not fully understood. As HOSCN reacts rapidly and specifically with thiols, we hypothesized that HOSCN, and the related oxidant hypochlorous acid (HOCl), may inhibit SERCA activity via Cys oxidation. Rat sarcoplasmic reticulum vesicles containing SERCA were incubated with pathological concentrations (0-100 μM) of MPO-derived oxidants for 2 h. This resulted in up to 75 % loss of SERCA activity with pre-formed HOSCN and HOCl, and 65 % and 50 % inhibition with MPO-generated HOSCN and HOCl, respectively. Decomposed HOSCN had no effect. This loss of activity occurred in parallel to a dose-dependent (> 50 % loss) of SERCA Cys residues. the study was extended to examine perturbations in $Ca^{2+}$ levels in human coronary artery endothelial cells (HCAEC) exposed to MPO oxidants. Exposure of isolated HCAEC, with or without external $Ca^{2+}$, to either HOSCN or HOCl resulted in dose-dependent increase in intracellular $Ca^{2+}$ under conditions that did not result in immediate losses in cell viability. Thapsigargin, a potent SERCA inhibitor, completely attenuated the intracellular $Ca^{2+}$ changes induced by HOSCN and HOCl, suggesting that these are SERCA-mediated. We conclude that MPO-mediated damage to SERCA, via Cys oxidation, may exacerbate endothelial dysfunction, a key early event in atherosclerosis.

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## VEGF-Induced Endothelial Cell Migration Requires Nox2 and Nox4-Dependent S-Glutathiolation of SERCA

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Endothelial cell (EC) migration in response to VEGF is a critical step in both physiological and pathological angiogenesis. Although VEGF signaling has been extensively studied, the mechanisms by which VEGF-dependent reactive oxygen species (ROS) production affects EC signaling are not well understood. the purpose of this study was to elucidate the involvement of Nox2 and Nox4-dependent ROS in VEGF-mediated EC $Ca^{2+}$ regulation and migration. VEGF induced migration of human aortic EC into a scratch wound over 6 hours and was inhibited by overexpression of either catalase or SOD. $H_2O_2$-stimulated EC migration also was inhibited by catalase, and unexpectedly by SOD. Both VEGF and $H_2O_2$ increased *S*-glutathiolation of SERCA2 and increased $Ca^{2+}$ influx into EC, and these could be blocked by overexpression of catalase or overexpression of SERCA2 in which reactive cysteine-674 was mutated to a serine. in determining the source of VEGF-mediated ROS production, our studies showed that specific knock down of either Nox2 or Nox4 inhibited VEGF-induced *S*-glutathiolation of SERCA, $Ca^{2+}$ influx, and EC migration. However, $H_2O_2$-induced *S*-glutathiolation of SERCA and $Ca^{2+}$ influx overcame the knockdown of Nox4 but not Nox2, suggesting Nox4 as the source of $H_2O_2$ . These results demonstrate that VEGF stimulates EC migration through increased *S*-glutathiolation of SERCA and $Ca^{2+}$ influx in a Nox2 and Nox4-dependent manner.

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