

Dynamic Response of the Livers of Chronic Ethanol-Treated Rats to Oxygenation Changes: an fMRI Study

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Introduction: The metabolism of alcohol by the liver causes increased O₂ demand and potentially decreased O₂ supply due to hepatic lipid accumulation and decreased blood flow through the narrow sinusoidal beds. This study addresses two questions: (1) can fMRI noninvasively assess oxygenation changes in the livers of living rats and (2) does chronic ethanol (CE) treatment alter oxygenation changes in livers of alcoholic rats.

Methods and Materials: The high-fat Lieber-DeCarli all-liquid diet (36% of calories as ethanol or dextrin-maltose) was administered to male Wistar rats for 6 - 8 weeks. For fMRI examination, rats were anesthetized with 2% isoflurane and mechanically ventilated. A T₂-weighted spin-echo fMRI protocol (TE 40 ms, TR 2.14s) provides relatively selective information about sinusoidal O₂ changes. A T₂*-weighted FLASH gradient-echo fMRI protocol (TE 15 ms, TR 154 ms, 20° RF pulse) provides more rapid (1.5 min) acquisition of images, although not selective for sinusoidal O₂ changes. Echo time (TE)-dependence studies of fMRI results were done on CE and pair-fed (PF) control rats during normoxia and then during carbogen challenge. A spin-echo (SE) multi-pulse sequence was used to determine T₂ dependence with echo times of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 msec. Each image was obtained as an 80 x 80 matrix, FOV of 8 x 8 cm, a slice thickness of 2.5 mm and with 4 transients per phase encoding step. For T₂* dependence, a single slice FLASH pulse sequence was used with echo times of 6, 8, 10, 14 and 18 ms. Two baseline images were acquired for all groups during normoxic ventilation and two images during hypoxic, hyperoxic and carbogen challenge, respectively. Blood oxygen content was determined after MRI examination but while the animals were still under inhalation challenge. The livers were exposed and 0.1 - 0.2 ml of blood was taken from the hepatic artery, portal vein and hepatic vein. Samples were analysed with a hemoximeter (OSM3 Hemoximeter Radiometer, Copenhagen) within 5 minutes of removal.

Results and Discussion: BOLD contrast in fMRI is based on deoxyhemoglobin being paramagnetic and decreasing T₂- and T₂*-weighted MRI intensities. Previously we showed that in spin-echo fMRI studies that PF control rats exhibited much greater changes in signal intensity than alcoholic rats during hypoxic (10% O₂), hyperoxia (98% O₂) or hypercapnia (5% CO₂, a vasodilator) challenge. MRI intensity changes correlated well with pulse oximetry measurements ($r^2 = 0.95$). Biochemical lactate / pyruvate ratios (cytoplasmic NADH / NAD⁺) also changed less with alcoholic vs. controls: hyperoxia: 9% vs. 31% decrease; carbogen: 7.7% vs. 38.8% decrease; hypoxia: 10.5% vs. 185% increase. Morphometric measurements showed that sinusoidal area decreased dramatically for alcoholic rats compared to controls, with sinusoidal area of 13.4% ± 2.25% for PF (mean ± SE, n = 7) compared to 4.2% ± 0.65% for CE rats (mean ± SE, n=7, statistically significant p<0.002). Blood oxygen content sampled from the portal vein, hepatic artery and hepatic vein show that CE rats have less oxygen in all three vessels than PF rats under normoxic, hypoxic, hyperoxic and carbogen challenge.

With gradient echo fMRI, chronic ethanol-treated rat livers also showed significantly slower rates (> 3min) of response and lower magnitude of response to hypoxia, hyperoxia and carbogen challenges relative to pair-fed controls (mean ± SE, n=5, p<0.05,): hyperoxia: 44% ± 3% vs. 14% ± 2% decrease; hyperoxia: 48% ± 4% vs. 27% ± 3% increase; carbogen: 79% ± 30% vs. 9% ± 3% increase. Thus, carbogen almost doubles the signal intensity for control rat liver, with almost no change in alcoholic rat liver. Echo time dependent studies of both spin echo and gradient echo fMRI ($\Delta S / S_0$ vs. TE) extrapolated roughly to zero for zero TE time under normoxic and carbogen challenge conditions. This indicates that these experiments are true Blood Oxygen Level Dependent (BOLD) effects dependent upon deoxyhemoglobin changes.

These results show that both SE and GE fMRI measurements reflect decreased oxygenation responsiveness in the livers of alcoholic rats to hypoxic, hyperoxic and carbogen challenge, compared to controls. This insensitivity in CE rats is due to decreased sinusoidal blood volume, reflecting accumulation of hepatic fat and denatured protein, and increased tissue pressure in alcoholic rats. The CE rats showed lower blood oxygen levels as well as less responsiveness to challenge. The 5-10 fold greater fMRI intensity changes in liver compared to the brain are due to higher hepatic blood volume (15-20% vs. 2-3%), higher microvascular blood content (60% of total liver blood vs. 33% in brain) and the decreased overall oxygenation of hepatic vs. brain blood.