# Mice liver metabolism and defence mechanisms under oxidative stress-related conditions:Hypotaurine as selective hepatic antioxidant?

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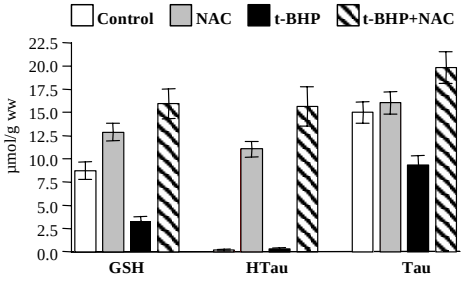
**Introduction**

Mitochondrial damage is being recognized as a key step in liver injury associated with oxidative stress. Mitochondria also have necessary roles in the genesis of apoptosis in certain cell types [1]. As mitochondrial function is tightly coupled to both production of free radicals and cellular energy production, changes in mitochondrial energy metabolism may be a central feature in liver injuries. Interestingly, a link between glucose metabolism and apoptosis has been reported recently [2]. The most widely used antidote to prevent oxidative damage to the liver is N-acetyl-cysteine (NAC), which is believed to exert its beneficial effect by the replenishment of the antioxidant glutathione (GSH). To clarify the involvement of hepatic intermediary metabolism in liver injury, we used *ex vivo* multinuclear NMR spectroscopy combined with *in vivo* injection of different $^{13}$C-labelled substrates 1) to characterize key metabolic pathways in mice liver at rest, 2) to investigate whether metabolic changes are associated with a) peroxide-mediated oxidative stress, b) oxidative stress due to mitochondrial impairment or c) anti-FAS mediated apoptosis, and 3) to adress whether NAC is involved in yet undescribed metabolic pathways of the liver under normal and oxidative-stress related conditions.

**Methods**

***Animal model.*** 1) BALB/C mice were injected with different $^{13}$C-labelled substrates ([U-$^{13}C_6$]glucose, [U-$^{13}C_3$]propionate, [1,2-$^{13}C_2$]acetate, [3-$^{13}C_1$]pyruvate, [3-$^{13}C_1$]alanine, [U-$^{13}C_2$]taurine; 2,2 mmol/kg, i.p.). 2) Mice were treated with a) tert-butylhydroperoxide (t-BHP; 50 mg/kg, i.p., 5 h), b) 3-nitropropionic acid (3-NPA; 50 mg/kg, i.p., 5 h), an inhibitor of succinic dehydrogenase, or c) anti-FAS antibody (0.5 µg/g; 1.5 - 7.5 h). 3) Mice were injected with NAC (100-600 mg/kg or 3 x 100 mg/kg; i.p.) alone or after treatment with t-BHP, 3-NPA or anti-FAS. 30 min after administration of the $^{13}$C-labelled substrates, the mice were killed by decapitation. The liver (and other organs for comparison) were removed and immediately snap-frozen in liquid nitrogen. ***Extraction.*** Tissue samples were powdered over liquid nitrogen and homogenized in perchloric acid (PCA) at 0°C [3]. To obtain lipid extracts from the same tissues, the pellets were extracted with $CHCl_3$/$CH_3OH$. Blood (taken from the neck) was immediately mixed with PCA and dual-extracted as well. ***NMR analysis***. After lyophilization, the samples were redissolved in 0.5 ml $D_2O$ (water-soluble metabolites) or in 0.8 ml $CDCl_3$/$CD_3OD$ (2:1) (lipid components) and centrifuged. 1D $^1$H-, $^{13}$C- and $^{31}$P-NMR spectra were recorded on Bruker spectrometers DRX 600 or AVANCE-NB/WB 360. Metabolite concentrations were calculated from $^1$H-NMR spectra; the percentage $^{13}$C-enrichments were calculated from $^{13}$C-NMR spectra as described previously [3]; the flux of $^{13}$C through metabolic pathways was followed up by $^{13}$C-isotopomer analysis of the $^{13}$C-$^{13}$C coupling pattern in amino acids. Gradient selected 2D-NMR inverse homonuclear (COSY) and heteronuclear (HSQC) correlations were applied to verify chemical shift data.

**Fig. 1. GSH, HTau and Tau concentrations in mice liver under control conditions and after treatment with t-BHP and/or NAC**




**Results**

**1)** Under physiological conditions, mitochondrial metabolism was most active using glucose, followed by propionate > acetate > alanine > pyruvate as $^{13}$C-labelled substrate. The major fraction of pyruvate from [U-$^{13}C_6$]glucose was metabolized anaplerotically via pyruvate carboxylase (PC; > 70%). While flux of carbon through glycolysis was low compared to the TCA cycle flux, the gluconeogenetic pathway led to considerable *de novo* synthesis of glucose and glycogen from propionate, followed by pyruvate > alanine > acetate. With the exception of glutamine, synthesized in the muscle from [1,2-$^{13}C_2$]acetate, only minor contributions of other organs to the labelling pattern of liver metabolites were detected. A significant *de novo* synthesis of glutathione (GSH) (the [$^{13}$C]glutamate residue of GSH was quantified from $^{13}$C-NMR spectra) was observed selectively in liver tissue, consistent with much higher basal concentrations of GSH (8.72 ± 0.93 µmol/g ww) compared to any other organ ($p<0.001$). **2)** After treatment of mice with either t-BHP or 3-NPA, oxidative stress and mitochondrial injury in the livers were observed as indicated by increased blood GSH levels concomitant to >60% decreased GSH tissue concentrations ($p<0.05$), >35% decreased taurine levels ($p<0.001$), >20% increased oxidation of polyunsaturated fatty acids (PUFA`s) ($p<0.01$), and >50% ATP depletion in parallel to > 60% impaired mitochondrial glucose oxidation (via pyruvate dehydrogenase; PDH) and TCA cycling ratio (as indicated by the ratio of the glutamate isotopomers [3,4,5-$^{13}C_3$] (2. PDH turn)/([4,5-$^{13}C_2$] (1. PDH turn) + [2,3,4,5-$^{13}C_4$] (1. PC turn)). **3)** Distinct changes were observed in the liver of mice treated with anti-FAS. Mitochondrial glucose metabolism, glycogen synthesis, as well as GSH concentrations significantly increased at early time-points (after 1.5 h) ($p<0.001$) with only minor changes of ATP. However, marked decrease of carbon flux through PDH and GSH depletion (to 29 ± 9.6% of control) were detected concomitant to the development of massive apoptotic morphology after 7.5 h. **4)** After NAC injection into control mice, liver GSH levels increased concentration-dependent by up to 136% of control ($p<0.05$). NAC treatment had no effect on taurine (14.98 ± 1.17 µmol/g ww in the liver), an amino acid, that can be synthesized from cysteine. Hypotaurine (HTau), the "reduced form" and direct precursor of taurine, was hardly detectable in all organs (0.08 ± 0.03 µmol/g ww in the liver). After administration of NAC, however, HTau increased selectively in the liver 150-fold ($p<0.001$). **5)** When NAC was investigated concomitantly with GSH-depletion due to t-BHP or 3-NPA, there was a significant attenuation of taurine and PUFA depletion ($p<0.05$), and almost complete recovery of mitochondrial energy metabolism ($p<0.001$). NAC did not influence blood GSH levels, but compensated for decreased tissue levels by stimulating *de novo* synthesis. In addition, HTau and Tau concentrations increased to 140% and 120% of NAC-treated controls, respectively. **6)** In anti-FAS treated mice, NAC significantly restored liver GSH levels ($p<0.001$) but increased HTau levels only slightly ($p<0.05$). Furthermore, under GSH depleted conditions, NAC stimulated key pathways involved in energy- and NADPH production (i.e. the pentose phosphate shunt as indicated by formation of ribose-5-phosphate).

**Conclusions**

The present data demonstrate the usefulness of *ex vivo* multinuclear NMR spectroscopy to study various aspects of liver intermediary metabolism associated with oxidative stress-associated events. Furthermore, changes in energy metabolism may explain some mechanisms involved in apoptosis. In particular, initially activated glucose metabolism and subsequent sustained energy depletion might trigger death receptor engagement by the provision of the needed energy and render the liver cells more susceptible to oxidative stress at later stages, respectively. Furthermore, the observed NAC-induced alterations in hepatic metabolism improve the understanding of its beneficial effect in liver injuries. We suggest that, apart from GSH replenishment, several other metabolic actions of NAC contribute to the maintainance of the liver`s normal redox state. In particular, formation of HTau, which can be oxidized to taurine, may represent a further antioxidant defence mechanism in the liver.

**References**

[1] Nieminen AL. Int Rev Cytol 2003; 224:29-55; [2] Danial et al. Nature 2003, 424: 952; [3] Zwingmann et al., Hepatology 2002, 37: 420