Non-invasive Analysis of Gallbladder Bile Composition in Cynomolgus Monkeys using In Vivo 1H-MRS

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Background - Bile is an important, though often neglected compartment in mammalian lipid and cholesterol metabolism. Numerous common diseases including obesity are associated with biliary malfunction and pathological changes in bile acid constitution that may readily lead to gallstone formation (1). Hence, repetitive assessment of bile composition would be of utmost interest for monitoring disease progression and efficacy of drug treatments. A large body of biochemical information on bile composition has been obtained with *in vitro* methods including ^{1}H-MRS (2). To date, however, biochemical analyses of bile still require invasive cannulation for bile sampling that have prevented widespread application. On the other hand, a single study recently reported *in vivo* ^{1}H-MR spectra of human bile, though with spectral quality insufficient for detecting biliary constituents other than phospholipids (3). The purpose of the present study was (i) to improve spectral quality of localised ^{1}H-MRS such as to enable non-invasive assessments of bile composition *in vivo*, and (ii) to apply it for the assessment of the inter- and intra-individual variations of bile composition in a cynomolgus monkey model which closely reflects human lipid and cholesterol metabolism.

Methods - Six male cynomolgus monkeys (7.9-9.3 kg) were housed in groups, except for feeding, and were kept under standardised nutritional conditions. For MR investigations, animals were anaesthetised with ketamine/climazolan (*i.m.*). Body temperature was maintained with an electric heating blanket, and breathing rate and breathing gases were continuously monitored. Each animal was assessed four times in weekly intervals. MR measurements were conducted on a Bruker Biospec 4.7T / 40cm instrument equipped with a curved rectangular (9cm x 9cm) surface coil for signal excitation and acquisition. Axial scout images were obtained using a multi-slice FLASH sequence (TR/TE=156/5 ms). Localised ^{1}H-MR spectroscopy was performed with a PRESS sequence with TR/TE=2000/20 ms, a volume-of-interest of (7 mm)^3, CHESS water suppression, 64 averages, and respiration gating.

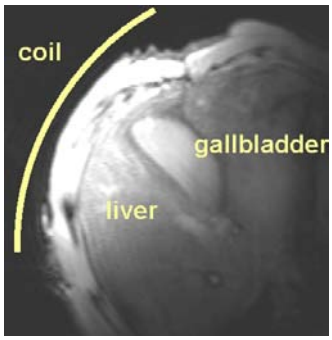

Fig. 1: *Axial FLASH image of monkey abdomen.*

Results and Discussion - Figure 1 depicts an axial scout image through the liver of a cynomolgus monkey. Rapid gradient-echo imaging provided good contrast of the gallbladder from surrounding liver tissue. Moreover, there was no layering of the gallbladder bile observed, thus enabling straightforward positioning of the volume-of-interest for ^{1}H-MRS. With these prerequisites in place, we obtained excellent *in vivo* ^{1}H-MR spectra of gallbladder bile, as shown in figure 2. Hitherto unmatched spectral quality allowed numerous different species of bile acids to be identified and quantified, including their glycine and taurine conjugates and phospholipids. In particular, specific hydroxylation patterns at positions H3, H7 and H12 of the cholesteryl scaffold with corresponding well-resolved resonances provided a handle to quantitatively ascertain cholate, chenodeoxycholate and deoxycholate as the major bile acids in bile of cynomolgus monkey. More than 90% of the bile acid pool was found to be conjugated either with glycine or taurine. These findings are in excellent agreement with *in vitro* studies carried out on bile obtained invasively (4).

Repeated assessment of six male cynomolgus monkeys in a combined cross-sectional and longitudinal study provided insight into the inter- and intra-individual variability of gallbladder bile composition. Marked inter-individual differences in bile acid configuration and conjugation were detected although all monkeys were on a standardised diet already one week prior to the first MR-examination. On the other hand, the intra-individual variability was smaller, thus indicating characteristic subject-related patterns of bile acid composition.

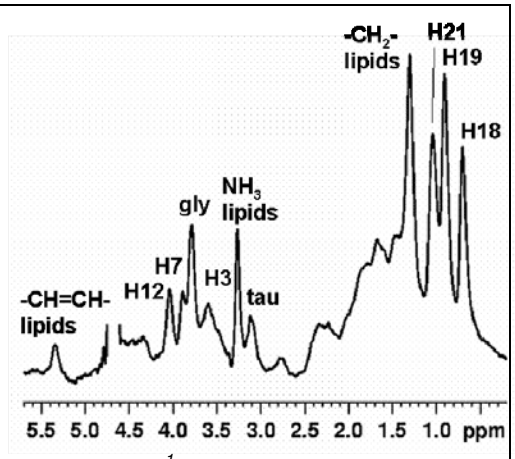

Fig. 2: *In vivo ^{1}H-MR spectrum of gallbladder bile in cynomolgus monkey.*

Conclusions - The present data demonstrate that quantitative high-resolution ^{1}H-MRS assessment of gallbladder bile is readily feasible in situ. Extension to pathological states thus opens up a new avenue for non-invasively and repetitively monitoring a further important compartment of lipid and cholesterol metabolism.

References

1. Moseley RH. *in* Textbook of gastroenterology. *eds:* Yamada T et al. Lippincott, Philadelphia (1995) Vol. 1, pp 383-404.
2. Barnes S *et al. in* The bile acids: chemistry, physiology, and metabolism. *eds:* Setchell KDR *et al.* Plenum Press, New York (1988) Vol 4, pp 65-137.
3. Prescot AP *et al.* Radiology 2003; 229:587-592.
4. Stephan ZF *et al.* Am J Clin Nutr 1981; 34:204-210.