Simultaneous Quantification of Glycine- and Taurine-conjugated Bile Acids, Total Bile Acids and Phospholipids in Human Bile by using ^{1}H MRS

O. B. Ijare[1], T. Bezabeh[1], N. Albiin[2], A. Bergquist[2], U. Arnelo[2], B. Lindberg[2], and I. C. Smith[1]
[1]National Research Council Institute for Biodiagnostics, Winnipeg, Manitoba, Canada, [2]Karolinska University Hospital, Karolinska Institutet, Huddinge, Stockholm, Sweden

INTRODUCTION: Bile acids, phospholipids, and cholesterol are the major lipid components in human bile [1]. The composition of bile is altered in various chronic cholestatic diseases and determining such alterations will be of great clinical importance in understanding the pathophysiology of these diseases [2]. Although there are a few chromatographic [3] and NMR-based methods [4,5] available for the quantification of glycine- and taurine-conjugated bile acids in bile, they are limited to *in vitro* applications. Recently, the feasibility of *in vivo* ^{1}H MRS of bile has been tested in humans [6] and cynomolgus monkeys [7]. In this study, we propose a robust method for the simultaneous quantification of glycine-conjugated bile acids (GCBAs), taurine-conjugated bile acids (TCBAs), total bile acids (TBAs) and phospholipids (PLs) in a single step, which could be extended to *in vivo* applications.

MATERIALS AND METHODS: Bile samples were obtained from patients (n=10) undergoing endoscopic retrograde cholangiopancreatography (ERCP) examination for various cholestatic diseases. The samples were collected by deep cannulation of the common bile duct (CBD) before the injection of the radiocontrast agent. 1D ^{1}H MR spectra were obtained for all bile samples on a 360 MHz spectrometer (Bruker Instruments). The peak areas of lipid signals were obtained by deconvolution (XWINNMR software) using 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TSP) as an external standard.

RESULTS & DISCUSSION: The bile acids in human bile are generally conjugated to the amino acids glycine and taurine. As a result, distinct and well-resolved amide proton (NH) signals can be observed in the downfield region of ^{1}H MR spectrum of bile (7.8 – 8.1 ppm). Previously, these signals have been used for the quantification of total glycine- and taurine-conjugated bile acids in human bile [5]. However, these amide protons are in dynamic exchange with the biliary water and their signals do not represent 100% intensity under physiologic conditions of pH. Thus, we changed the pH of the bile to slightly below the physiologic pH, to 6 ± 0.5 [5]. Since this approach is only applicable to *in vitro* studies, we herein propose an alternative approach to use the glycine- and taurine-methylene (CH_2) signals which are conjugated to bile acids for the quantification of glycine- and taurine-conjugated bile acids. TBAs and PLs have also been quantified along with GCBAs and TCBAs.

Figure 1 shows the ^{1}H MR spectrum of human bile along with its deconvoluted counterpart, showing the metabolite signals used for the quantification of various lipid components. In the ^{1}H MR spectrum of bile, the glycine-methylene protons resonate at ~3.73 ppm. These signals partially overlap with signals from other biliary lipids such as choline-CH_2 signals of PLs. Thus, it is difficult to measure the peak area of the glycine-CH_2 signal by manual integration. However, the peak area of this signal could be determined by deconvolution and used for the quantification of GCBAs.

Similarly, taurine conjugated to bile acids has two methylene proton signals resonating at 3.07 and 3.56 ppm. The signal at 3.56 ppm overlaps the other bile acid signals and is not accessible for quantification purposes, whereas the signal at 3.07 ppm is well resolved and has been utilized for the quantitation of TCBAs by us and others previously [4,5]. This signal has been utilized in the quantification of TCBAs in this study.

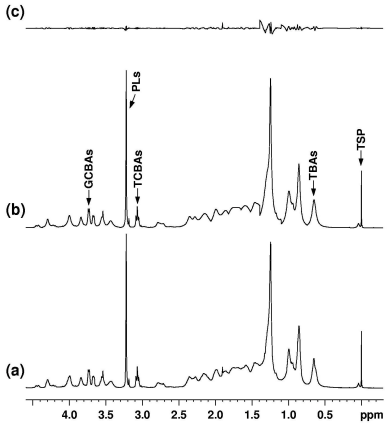

Figure 1: ^{1}H MR spectrum of bile (a) along with its deconvoluted counterpart (b) showing the marker lipid signals. Figure 1(c) is the difference of (a) and (b).

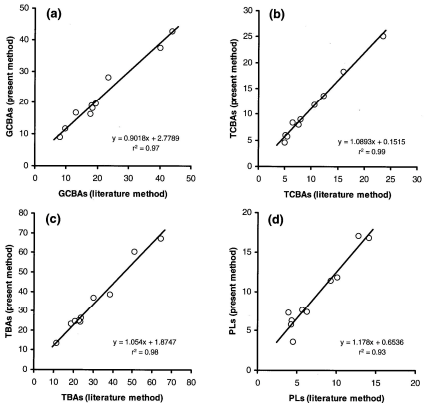

Figure 2: Plots of the quantities of GCBAs, TCBAs, TBAs and PLs determined in bile by the present method vs. those obtained using a literature method [ref. 8].

The H-18 methyl signals of bile acids and cholesterol resonate together around 0.65 ppm and their peak areas should be a measure of total bile acids and cholesterol. However, in this study, we observed that the sum of the total glycine- and taurine-conjugated bile acids determined using their methylene signals (CH_2) was almost equal to the sum of total bile acids and cholesterol determined from the H-18 methyl signal. This observation indicates that the total bile acid pool in bile is almost completely conjugated to glycine and/or taurine, and the contribution of cholesterol to the H-18 signal is minimal, which could be attributed to the rigidity of the cholesterol-phospholipid vesicles. This results in reduced T_2-relaxation time of cholesterol causing an extended broadening of its signals leading to the disappearance of cholesterol signals from ^{1}H NMR spectra of bile. Thus, H-18 methyl signals could in fact be used for the quantitation of TBAs in bile.

The levels of PLs in bile have been quantified using the peak area of their choline-$N^+[(CH_3)_3]$ signal resonating at 3.22 ppm [6]. The peak areas of all lipid signals (Fig.1) could be obtained simultaneously by deconvolution, making the present method fast and robust. We compared the results of the present method with an NMR-based literature method [8], in which bile has to be dissolved in polar organic solvent, such as DMSO, limiting its *in vivo* utility. We obtained a good correlation between both methods (Figure 2). The observed regression coefficients (r^2) for GCBAs, TCBAs, TBAs, and PLs were 0.97, 0.99, 0.98 and 0.93 respectively.

CONCLUSION: The proposed method has a potential for the simultaneous quantification of various lipid components in bile. *In vivo* ^{1}H MRS of bile has been shown to be feasible, and such a methodology could be extended to *in vivo* applications using clinical scanners which will be of value in the non-invasive diagnosis of cholestatic diseases.

REFERENCES:

1. Hay DW et al., *Hepatology* 1990; **12**:6S-14S.
2. Albiin N et al., *Acta Radiol* 2008; **49**:855-862.
3. Perwaiz S et al., *J Lipid Res* 2001; **42**:114-119.
4. Ishikawa H et al., *J Lipid Res* 1999; **40**:1920-1924.
5. Ijare OB et al., *Magn Reson Med* 2005; **53**:1441-1446.
6. Prescot AP et al., *Radiology* 2003; **229**:587-592.
7. Kunnecke B et al., *Biochim Biophys Acta* 2007; **1771**:544-549.
8. Nagana Gowda GA et al., *Lipids* 2006; **41**:577-589.