EXPLORING URINARY BILE ACIDS AS POTENTIAL MARKERS OF METABOLISM: REFERENCE VALUES IN CHILDREN BY TARGETED LC-MS/MS

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INTRODUCTION

Bile acids (BA) are C_{24} steroids synthesized in liver from cholesterol[1]. They can be conjugated by amidation[1] and sulfation[2].

While BA's role as emulsifiers has been known for long, their additional endocrine functions have lately aroused interest[3].

In comparison to BA in blood, it is surprising that hardly any data exist on BA in the most accessible human biofluid urine – especially when it comes to children.

AIM

- Development and validation of a **targeted LC-MS/MS method** for measurement of 18 urinary BA
- Establishment of **reference values** for urinary BA in healthy children aged 3-18 years

METHOD

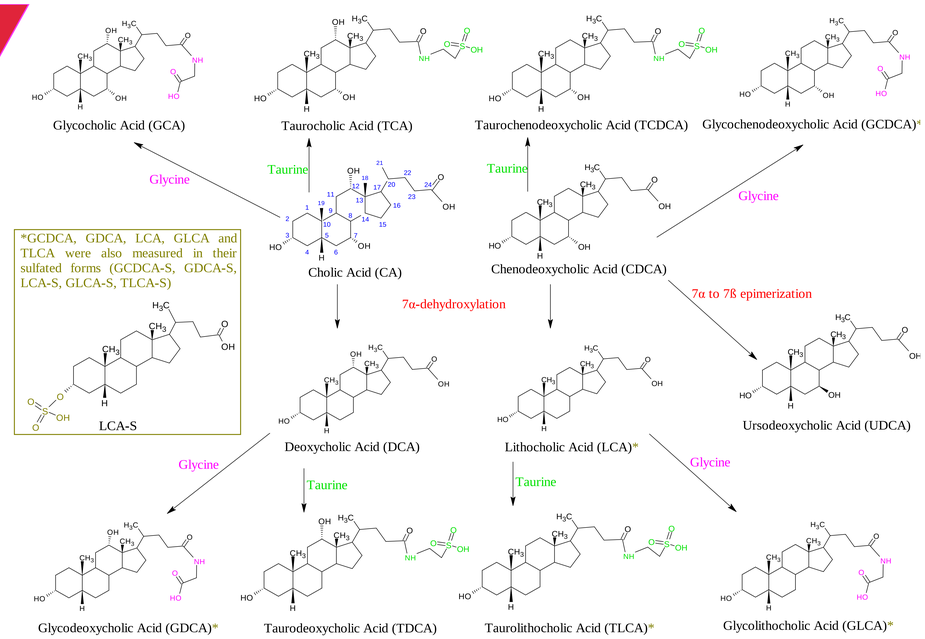
2 mL of 24-hour urine were used for sample preparation comprising protein precipitation (acetonitrile-$ZnSO_4$) and solid phase extraction (C18 cartridges).

Reversed-phase liquid chromatography was done on a phenyl-hexyl column, followed by tandem mass spectrometry with a triple quadrupole mass spectrometer using electrospray ionization (ESI) in the negative mode.

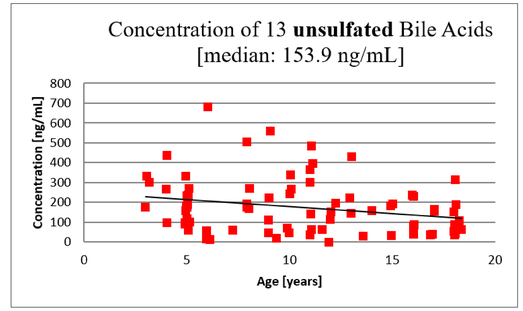
RESULTS

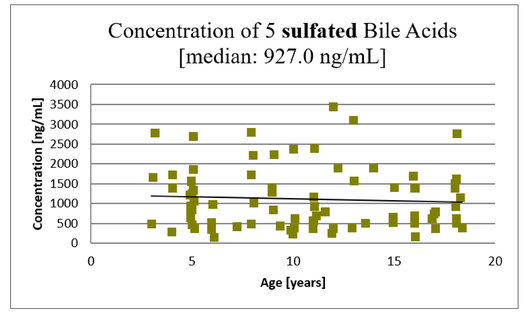
The method achieved good linearity (R^2 > 0.99) and recovery (90.49% - 113.99%). Intra-day/inter-day precision and accuracy ranged from 0.42% to 11.47% and 85.75% to 110.99%, respectively. No significant matrix effect was observed.

CA (median: 55.2 ng/mL) and GCA (48.9 ng/mL) were the two dominant non-sulfated BA. However, sulfated BA showed much higher concentrations, with GCDCA-S (337.5 ng/mL) showing the highest levels among all BA, followed by GLCA-S (197.4 ng/mL) and GDCA-S (183.2 ng/mL). In total, 86.5% of quantified BA were sulfated. The total concentrations of glycine amidated BA measured were higher than taurine amidated and non-amidated ones. No obvious trends between urinary BA and age or sex, respectively were observed.



Chemical structures of the 18 analyzed BA: The presented 13 BA plus 5 BA sulfates (see small box) can be measured with our targeted LC-MS/MS method.





The median concentration of the group of sulfated BA was markedly higher compared to the median of the group of unsulfated BA.

CONCLUSIONS

- A new method for measuring 18 BA by targeted LC-MS/MS was successfully developed, validated and applied to 24-hour urine samples of 80 healthy children.
- Urinary BA concentrations neither changed with age nor showed a sex difference.
- BA were mostly present in their sulfated form in children's urine, indicating hepatic sulfation to be a major metabolic pathway for urinary BA excretion in humans.

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