CORTOIC ACIDS: RENAISSANCE OF A FORGOTTEN CLASS OF STEROIDS

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INTRODUCTION

The C21 steroidal acids (**cortoic acids**) (α-)cortolic acid, β-cortolic acid, (α-)cortolonic acid and β-cortolonic acid present the oxidative end products of cortisol metabolism[1,2]. They have been assumed to constitute up to 25% of total urinary cortisol metabolites[1-5].
However, their analysis has been difficult[3,6], few data has been published in adults, and this class of steroids has become practically forgotten. Data in children are lacking completely.

AIM

- Developing a **practical analytical method** for quantification of urinary cortoic acids
- Establishing **reference values** for urinary cortoic acids excretion in healthy children

METHOD

5 ml aliquots of 24-hour urine samples were used. Sample work up consisted of solid phase extraction (C18 cartridges), strong anion exchange and derivatization.

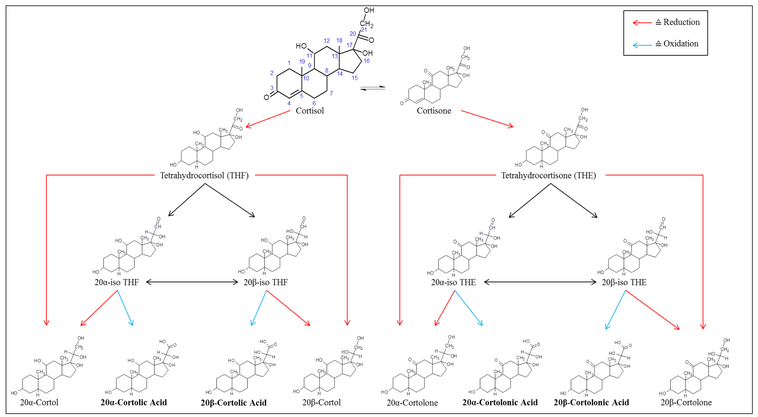
Cortoic acids were measured as 2-propylester-trimethylsilylether derivatives.

The quantification was done by targeted GC-MS using a nonpolar GC column.

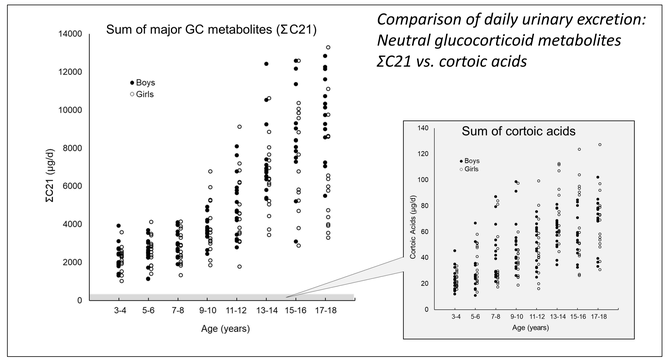
RESULTS

Baseline separation of all four cortoic acids was achieved on a nonpolar GC column. This enables a simultaneous sample work up and gas chromatographic determination of neutral and acidic cortisol metabolites – not in the same GC run, but with the same instrumental setup.
Calibration graphs were linear ($R^2 > 0.98$). Variations in precision and accuracy were less than 15%, respectively. The detection limit was 100 pg (injected).

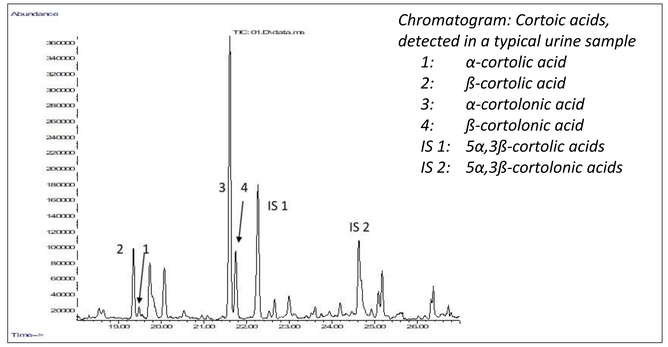
When excretion rates of the four cortoic acids were summed, all subjects excreted less than 130 µg/d (range: 11.0 µg/d - 127,3 µg/d). **Cortoic acids only represented about 1%** (range: 0,69% - 1,51) of total urinary cortisol metabolites. While **higher age led to increased excretion rates**, the children's sex did not significantly affect the amount of daily excreted cortoic acids.



Preferred biosynthetic pathway of cortoic acids according to Monder and Bradlow (1980)[2]



Comparison of daily urinary excretion: Neutral glucocorticoid metabolites ΣC21 vs. cortoic acids



Chromatogram: Cortoic acids, detected in a typical urine sample
1: α-cortolic acid
2: β-cortolic acid
3: α-cortolonic acid
4: β-cortolonic acid
IS 1: 5α,3β-cortolic acids
IS 2: 5α,3β-cortolonic acids

CONCLUSIONS

- Successful development, evaluation and application of a new and less complicated method for quantification of urinary cortoic acids using GC-MS
- Establishment of reference values by using data from 240 healthy children, adolescents and young adults
- Excretion of cortoic acids increased with age.
- **Cortoic acids' share in total urinary cortisol metabolites only added up to about 1%, a percentage much lower as hitherto estimated.**

REFERENCES

[1] **Bradlow HL, Zumoff B, Monder C, Lee HJ, Hellman L**. Isolation and identification of four new carboxylic acid metabolites of cortisol in man. *Journal of Clinical Endocrinology and Metabolism*. 1973;37(5):811-818. doi:10.1210/jcem-37-5-811

[2] **Monder C, Bradlow HL**. Cortoic Acids: Explorations at the frontier of corticosteroid metabolism. *Recent Progress in Hormone Research*. 1980;36:345-400.

[3] **Shackleton CHL, Roitman E, Monder C, Bradlow HL**. Gas chromatographic and mass spectrometric analysis of urinary acidic metabolites of cortisol. *Steroids*. 1980;36:289-298.

[4] **Monder C, Bradlow HL**. Carboxylic Acid Metabolites of Steroids. *Journal of Steroid Biochemistry*. 1977;8:897-908.

[5] **Aerts J**. Quantification of a glucocorticoid profile in non-pooled samples is pivotal in stress research across vertebrates. *Frontiers in Endocrinology*. 2018;9(OCT). doi:10.3389/fendo.2018.00635

[6] **Iohan F, Vincze I, Monder C**. High-Performance Liquid Chromatographic Determination of Cortolic and Cortolonic Acids as Pyrenyl Ester Derivatives. *Journal of Chromatography B: Biomedical Sciences and Applications*. 1991;564(1):27-41. doi:10.1016/0378-4347(91)80067-M

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