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Recent Advances in LC-MS/MS Analysis of Ancient Hormones

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RECENT ADVANCES IN LC-MS/MS ANALYSIS OF ANCIENT HORMONES

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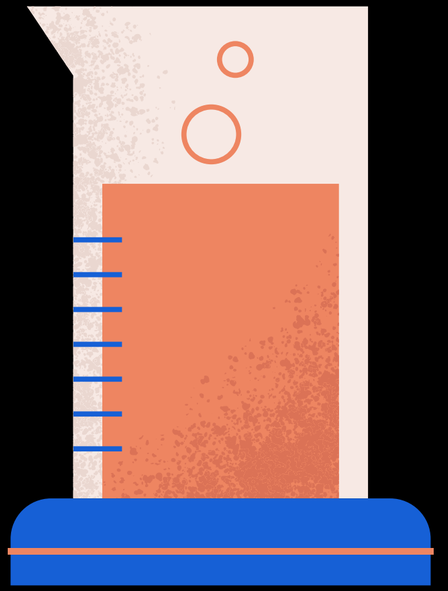

PREVIOUS RESEARCH

- Hair
- Dentine

Contents lists available at ScienceDirect

Journal of Archaeological Science

journal homepage: http://www.elsevier.com/locate/jas

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Emily Webb [a,*], Steven Thomson [b], Andrew Nelson [a], Christine White [a], Gideon Koren [b,c,d,h], Michael Rieder [b,c,d,e,f,g], Stan Van Uum [c,e]

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Bone, hair, dentine, fingernails

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Assessing relationship between tissues AND individuals

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Assessing relationship between tissues AND individuals

Population Variation

Studying how steroids differ between populations

MATERIALS, METHODS & ETHICS

Arnhem

Post-medieval (1626-1850 CE) Dutch collection
Permission for destructive analysis was provided by the local community and ethical clearance was granted from Leiden University.

Sex/Age

Standard pelvic and cranial traits were used for the estimation of sex and age.

MATERIALS, METHODS & ETHICS

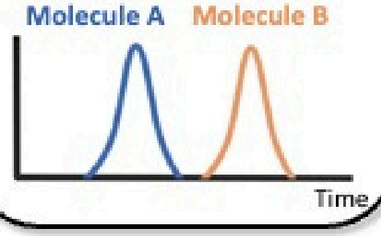
Steroid Extraction

1. HCl: 200mg, demineralize in 100mL 0.01 N HCl (Longin, 1971), 1mL MeOH (Van Uum et al., 2008)
2. EDTA: 200mg, demineralize in 10% EDTA (Tuross, 2012), 1mL MeOH
3. Ground Bone: 200 mg, 1mL MeOH
4. MeOH: 1mL MeOH (Charapata et al., 2018)

Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS analysis)

Liquid chromatography (LC) from Shimadzu

Molecules are separated based on chemical properties (polarity) in a chromatographic **column**



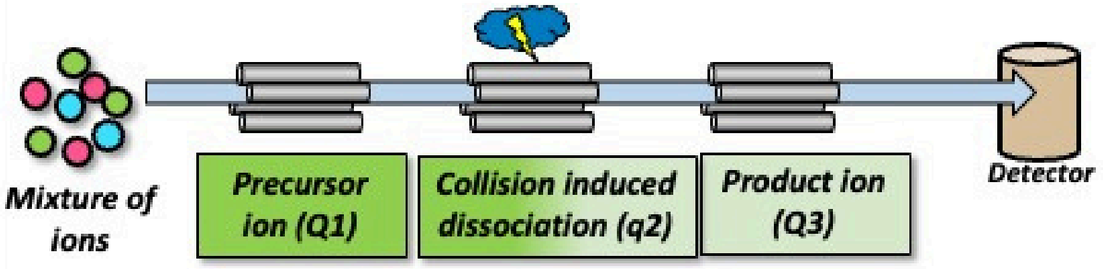
+

Mass spectrometer (MS) with a Triple Quadrupole (QqQ) analyzer (QTrap 6600+ from Sciex)

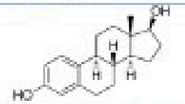
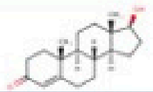
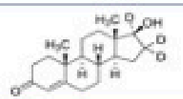
This combination offers **great sensitivity** and **selectivity** with **absolute quantification**

Molecules coming from **LC (liquid phase)** are **ionized (gas phase)** in the **MS**.
A combination of **three quadrupoles** leads to:

- **Isolation** of each molecule based on its mass-to-charge ratio (*m/z*) (in Q1)
- **Fragmentation** (in q2), and
- **Isolation** of a **specific fragment** coming from the parent ion (Q3)



Q1 → Q3 transitions are monitored for each steroid of interest.
A mixture of isotopically labeled compounds (cortisol-d4, testosterone-d3 and progesterone-d9) are used to account for losses during sample preparation and to ensure identification of the steroids of interest

Steroid	Structure	Nominal mass (Da)	Protonated ions (*m/z*)	Q1 → Q3 transitions
Cortisol		362	363.2	363.3 → 121.1
β-estradiol		272	273.1	237.1 → 107.0
Testosterone		288	289.2	289.1 → 97.0
Progesterone		314	315.2	315.1 → 109.0
Cortisol-d_4		366	367.2	367.1 → 121.1
Testosterone-d_3		291	292.2	292.1→ 97.0
Progesterone-d_9		323	324.2	324.1 → 100.0

Method adapted from previous publication: Koorneef et al. 2022 Front. Endocrinol. 13:960279

RESULTS

1. HCl demineralization rib (200mg)
2. EDTA deminerlizatoin rib (200mg)
3. Ground bone rib (200mg)

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4. MeOH extraction femur cortical (200mg)

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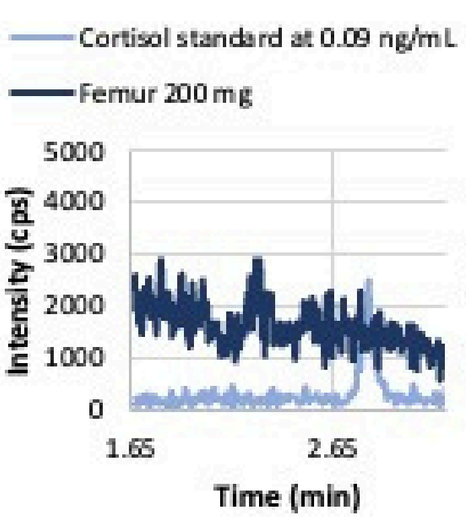
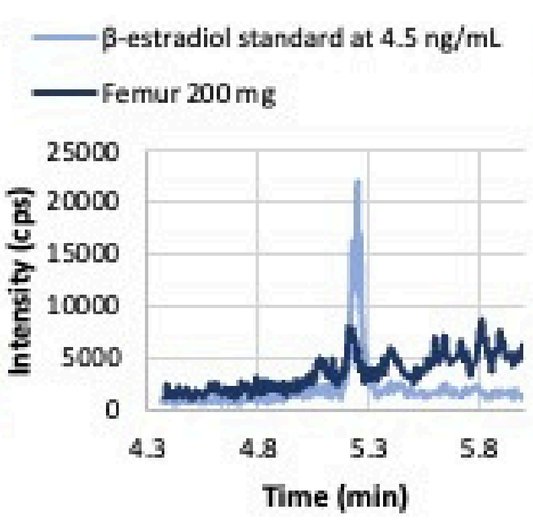
RESULTS

1. ~~HCl demineralization rib (200mg)~~
2. ~~EDTA deminerlizatoin rib (200mg)~~
3. ~~Ground bone rib (200mg)~~
4. MeOH extraction femur cortical (200mg)
5. MeOH extraction femur cortical (300-400mg)
6. MeOH rib (50, 100 mg)
7. MeOH femur cortical (50, 100, 200mg)

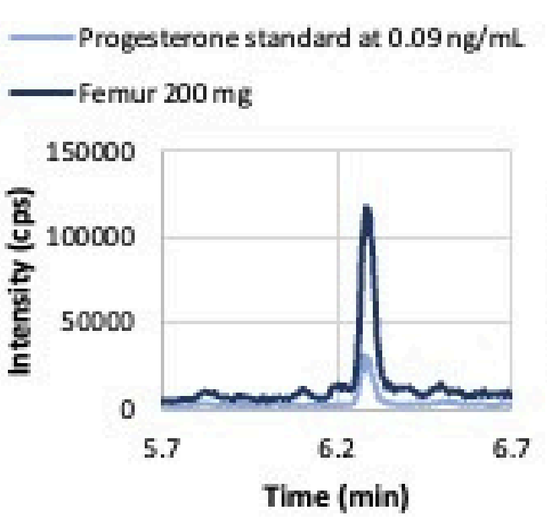
Cortisol levels in femur



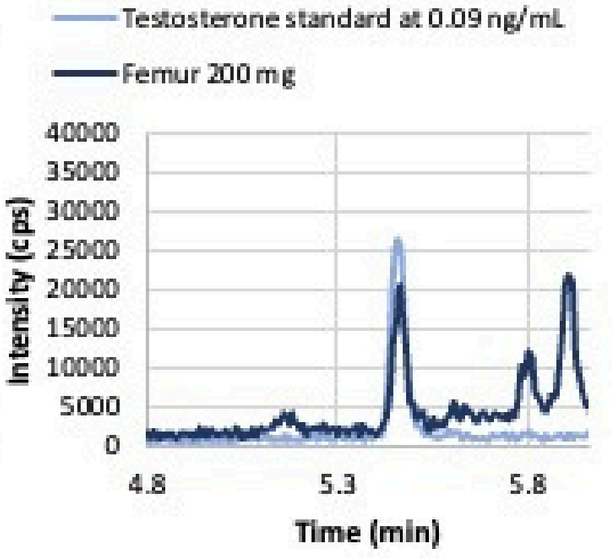
β-estradiol levels in femur



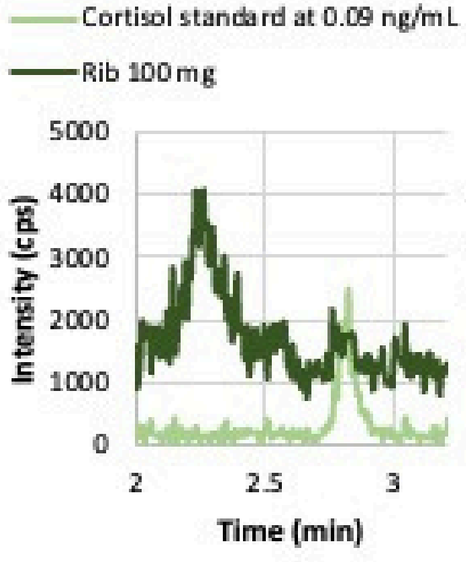
Progesterone levels in femur



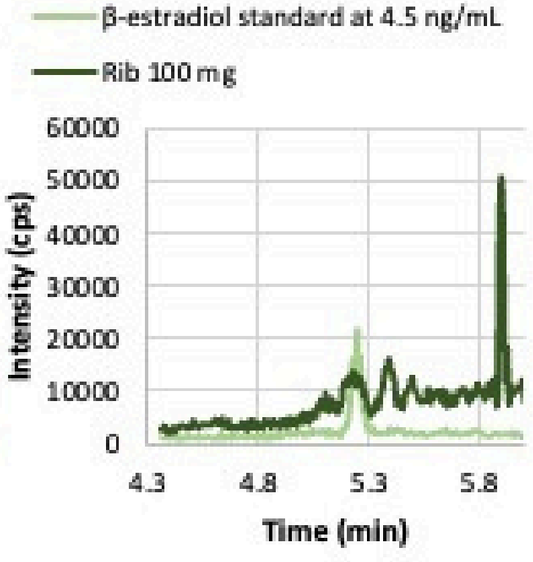
Testosterone levels in femur



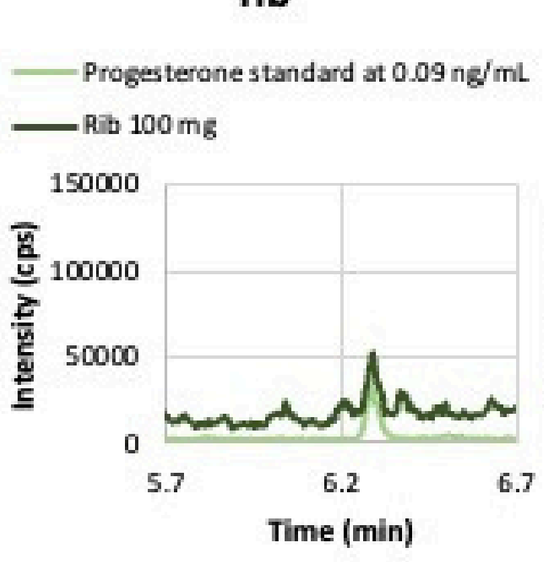
Cortisol levels in rib



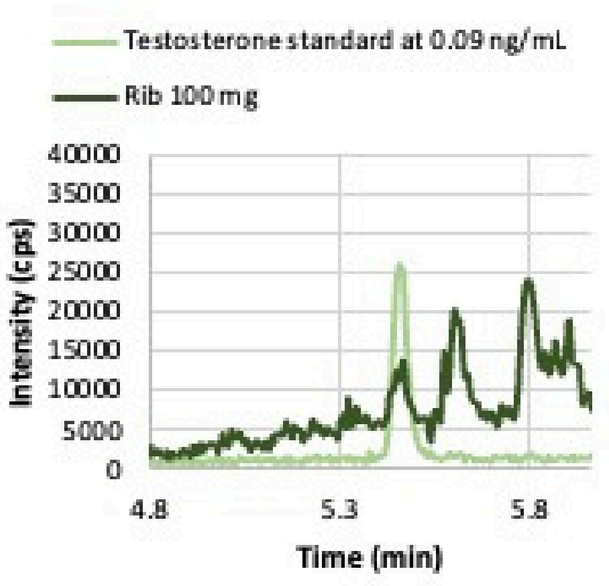
β-estradiol levels in rib

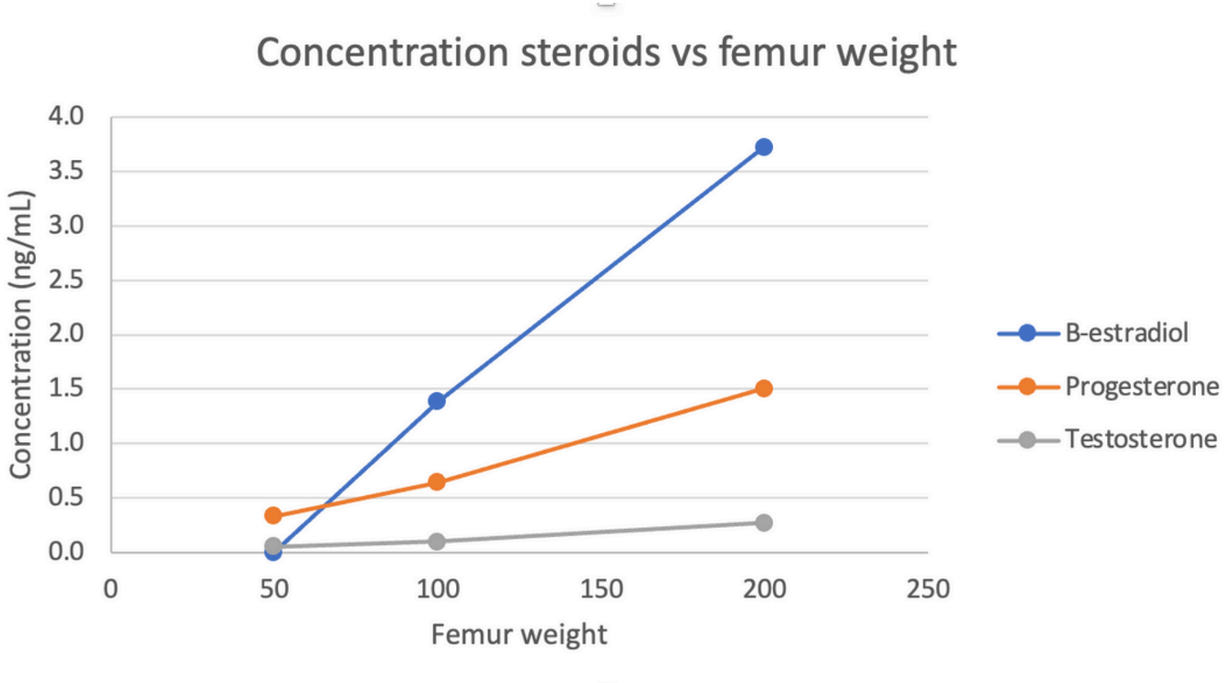


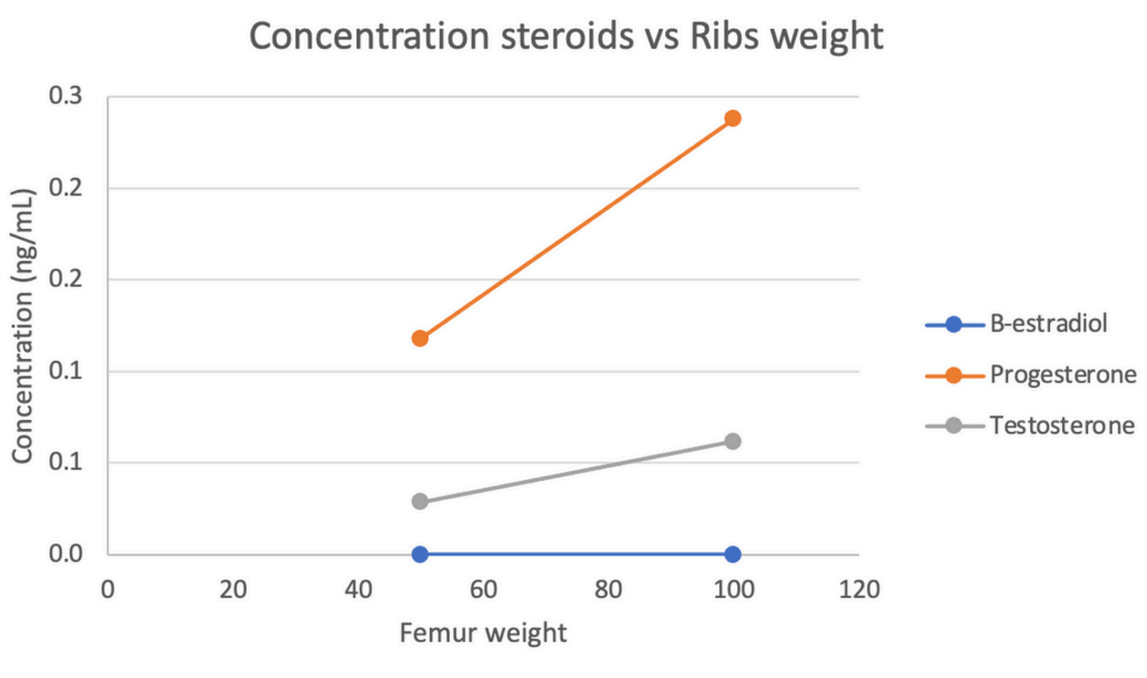
Progesterone levels in rib



Testosterone levels in rib




Concentration steroids vs femur weight
Concentration (ng/mL)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
250
Femur weight
B-estradiol
Progesterone
Testosterone


Concentration steroids vs Ribs weight
Concentration (ng/mL)
0.3
0.2
0.2
0.1
0.1
0.0
0
20
40
60
80
100
120
Femur weight
B-estradiol
Progesterone
Testosterone

SUMMARY

Cortisol

Cortisol does not appear to be present/detectable in femoral cortical or rib bone

Other Steroids

Testosterone, estradiol, and progesterone can all be reliably quantified

Implications

Paleohormonal analysis of archaeological material

NEXT STEPS

1. Intra-/Inter- individual variation
2. Sex/Age Variability
3. Other tissues
4. Other populations

THANK YOU!

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@DRSARAHSCHRADER

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