Universiteit Leiden
The Netherlands

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

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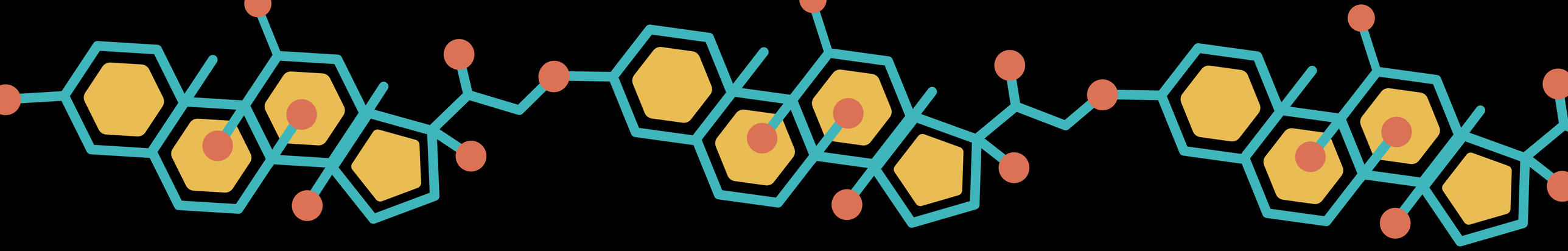

RECENT ADVANCES IN LC-MS/MS ANALYSIS OF ANCIENT HORMONES

Sarah Schrader,[1] Kevin Brewster,[2] Rachael Hall,[1] Martin Giera,[2] Elena Sánchez-López[2]

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06 Next Steps

PREVIOUS RESEARCH

- Hair
- Dentine

Contents lists available at ScienceDirect

Journal of Archaeological Science

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

Assessing individual systemic stress through cortisol analysis of archaeological hair

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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BRIEF COMMUNICATION

American Journal of Physical Anthropology

WILEY

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Elisha Tisdale[a,*], Lana Williams[a], John J. Schultz[a,b], Sandra M. Wheeler[a]

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THE EVOLUTION OF STRESS

LC-MS/MS

Higher sensitivity, with accuracy, reliability, and precision measurements

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Multi-Tissue & Lifecourse Appraoch

Bone, hair, dentine, fingernails

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Inter-/Intra- Individual Variation

Assessing relationship between tissues AND individuals

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Multi-Tissue & Lifecourse Appraoch

Bone, hair, dentine, fingernails

Inter-/Intra- Individual Variation

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

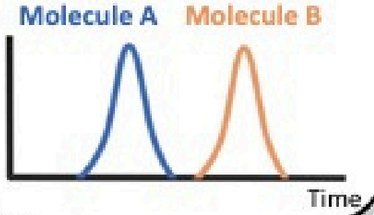
Steroid Extraction

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**



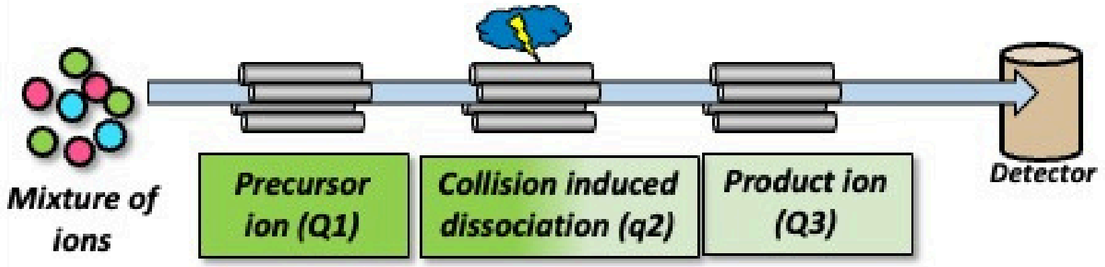
+

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

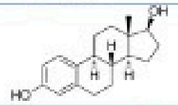
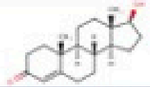
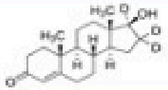
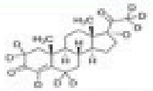
Mass spectrometer (MS) with a Triple Quadrupole (QqQ) analyzer (QTrap 6600+ from Sciex)

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

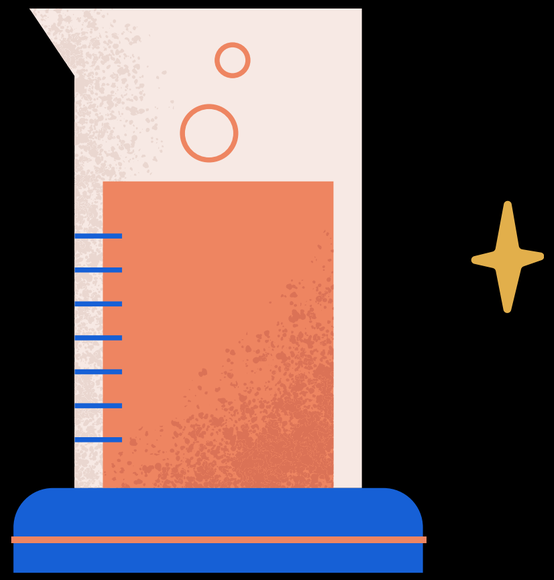
- **Isolation** of each molecule based on its mass-to-charge ratio (*m/z*) (in Q1)
- **Fragmentation** (in q2), and
- **Isolation** of a **specific fragment** coming from the parent ion (Q3)

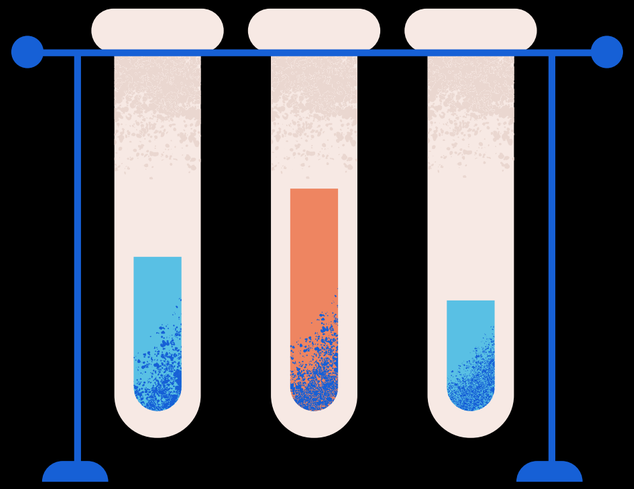


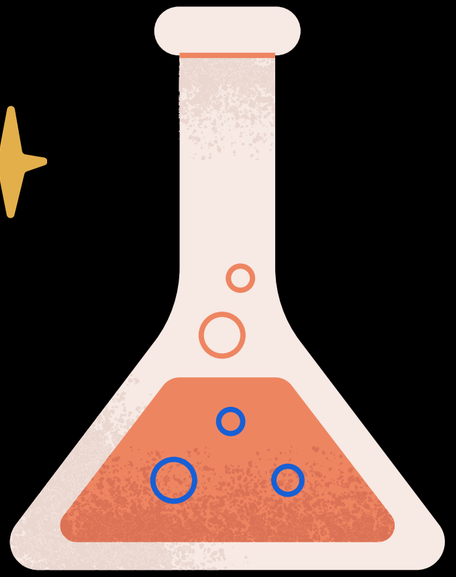
Q1 → Q3 transitions are monitored for each steroid of interest.
A mixture of isotopically labeled compounds (cortisol-d4, testosterone-d3 and progesterone-d9) are used to account for losses during sample preparation and to ensure identification of the steroids of interest

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

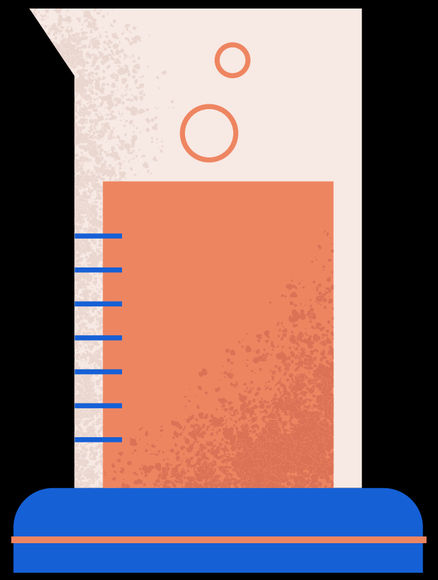


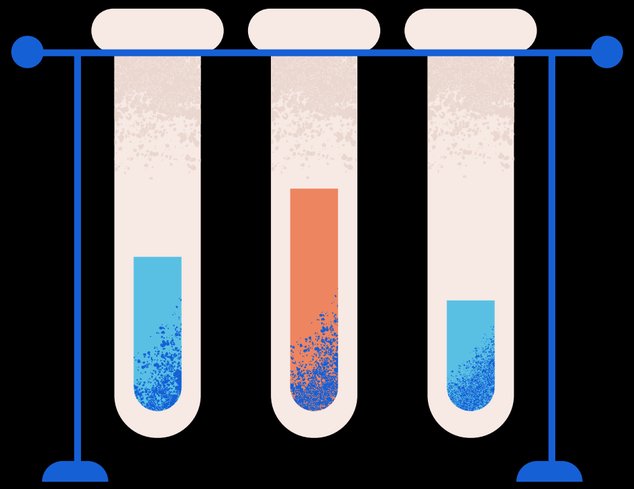




RESULTS

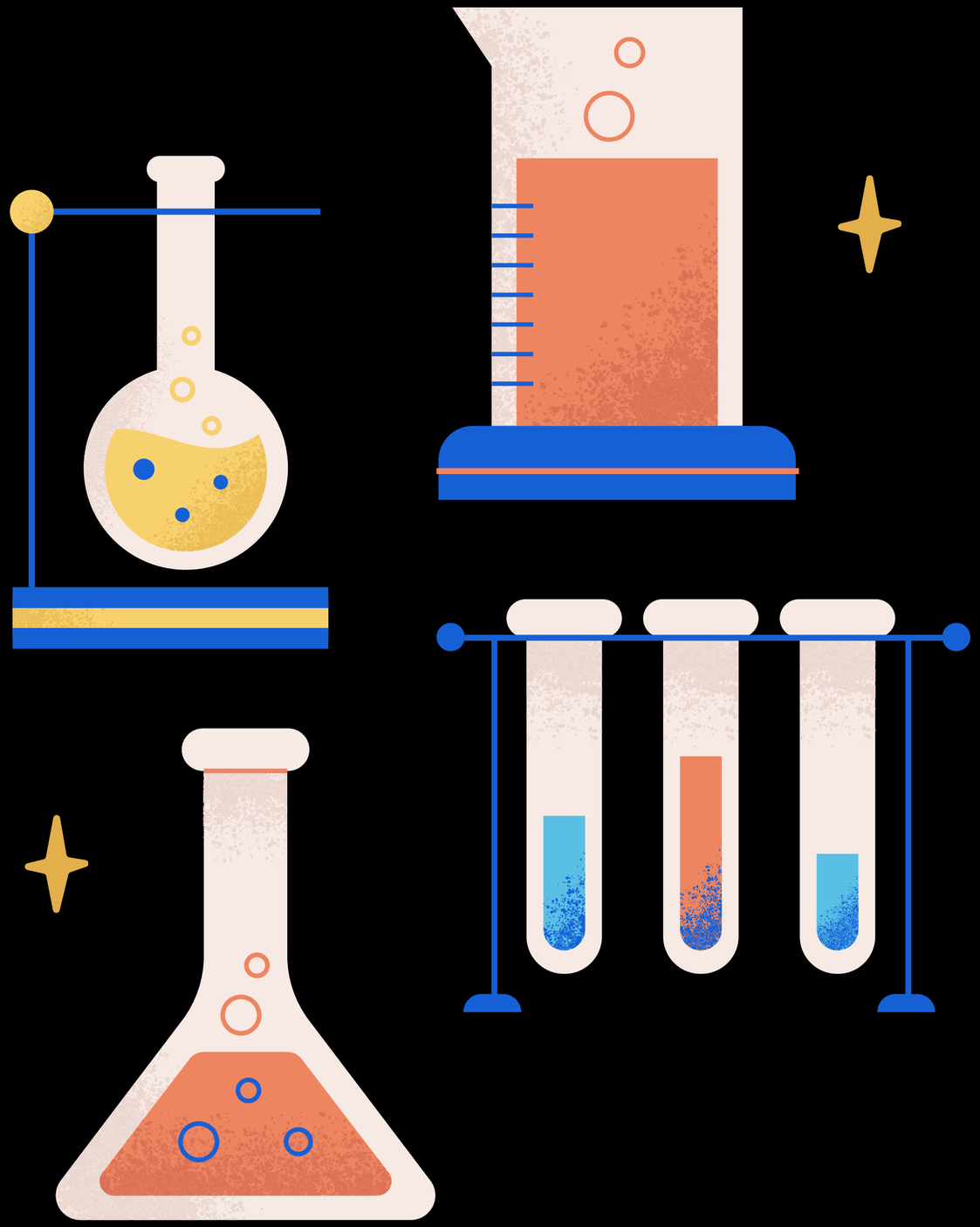
1. HCl demineralization rib (200mg)
2. EDTA deminerlizatoin rib (200mg)
3. Ground bone rib (200mg)





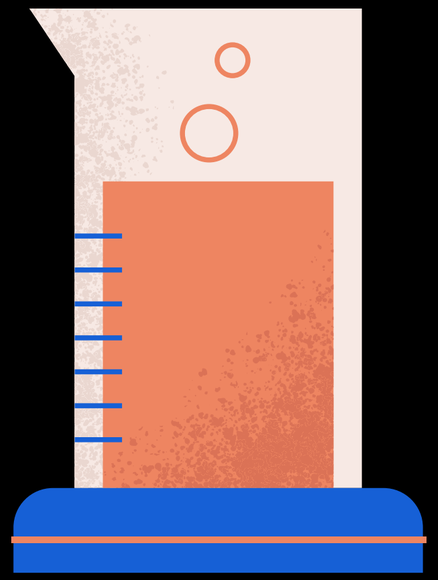
RESULTS

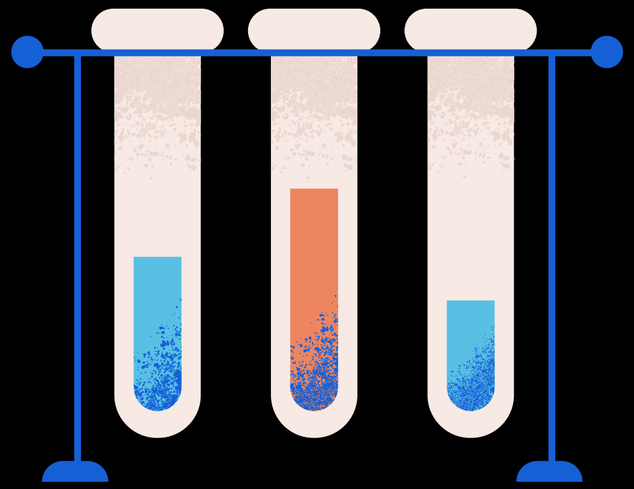
1. ~~HCl demineralization rib (200mg)~~
2. ~~EDTA deminerlizatoin rib (200mg)~~
3. ~~Ground bone rib (200mg)~~



RESULTS

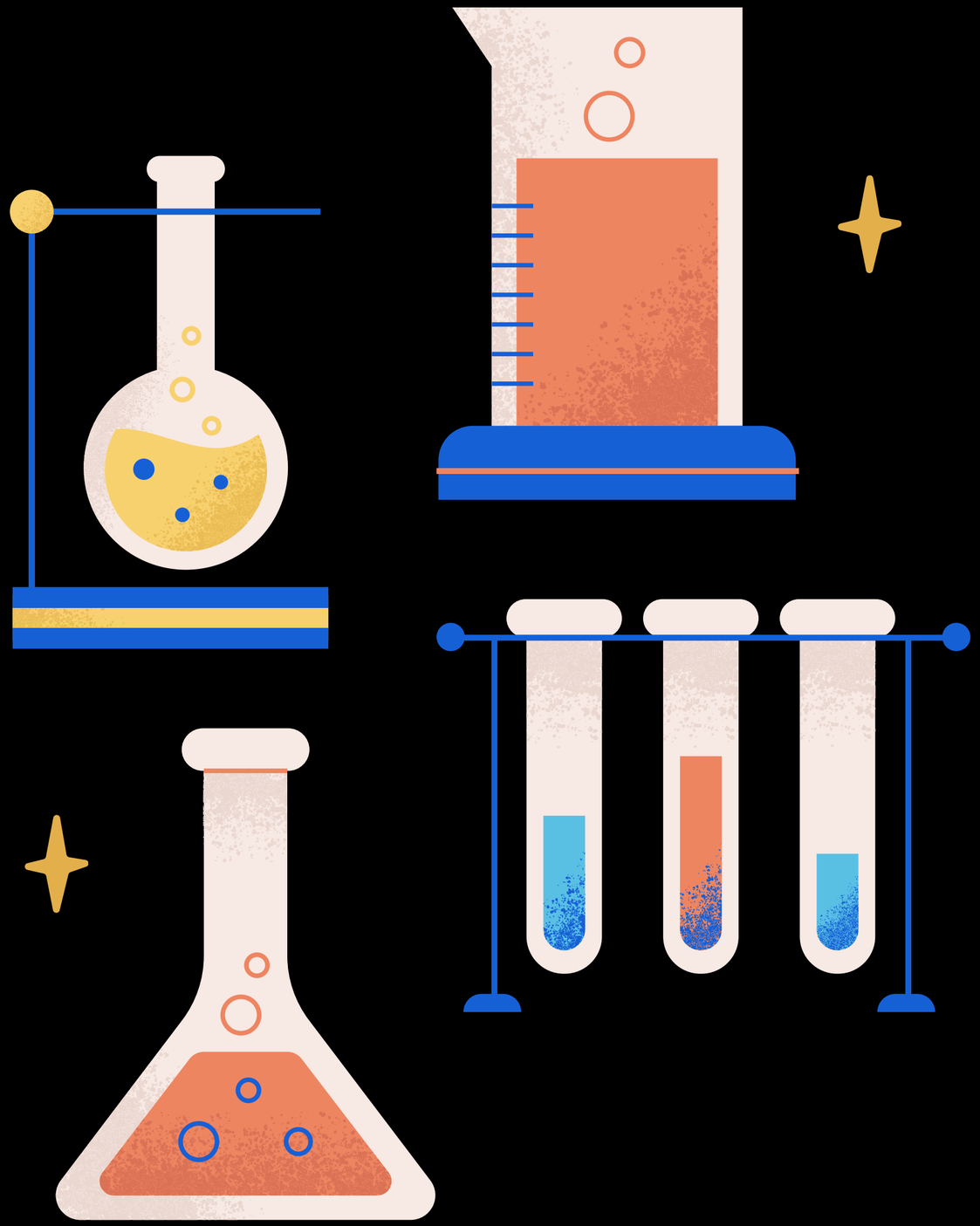
1. ~~HCl demineralization rib (200mg)~~
2. ~~EDTA deminerlizatoin rib (200mg)~~
3. ~~Ground bone rib (200mg)~~
4. MeOH extraction femur cortical (200mg)





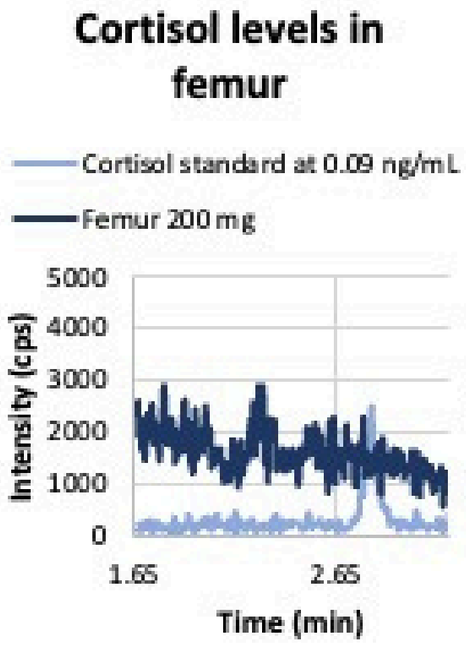
RESULTS

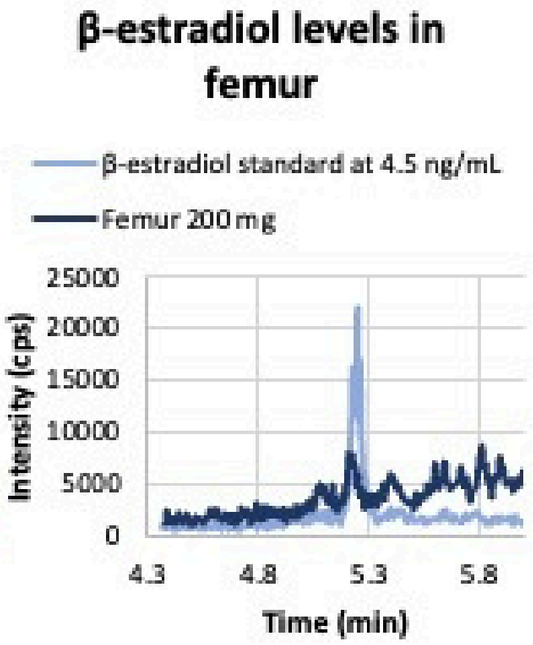
1. ~~HCl demineralization rib (200mg)~~
2. ~~EDTA deminerlizatoin rib (200mg)~~
3. ~~Ground bone rib (200mg)~~
4. ~~MeOH extraction femur cortical (200mg)~~

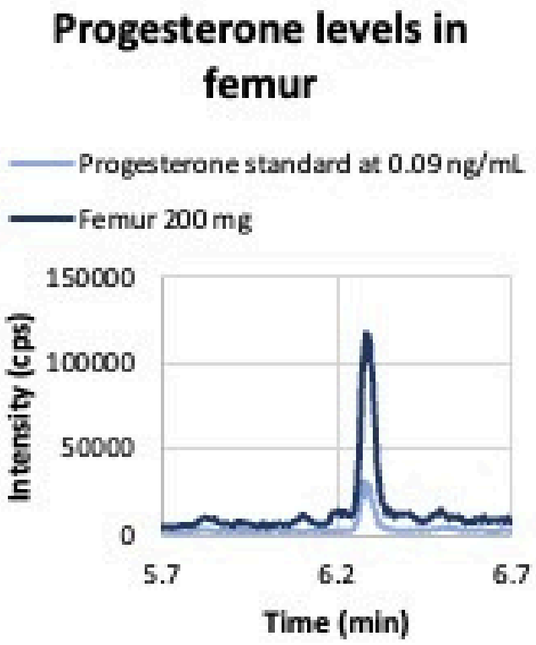


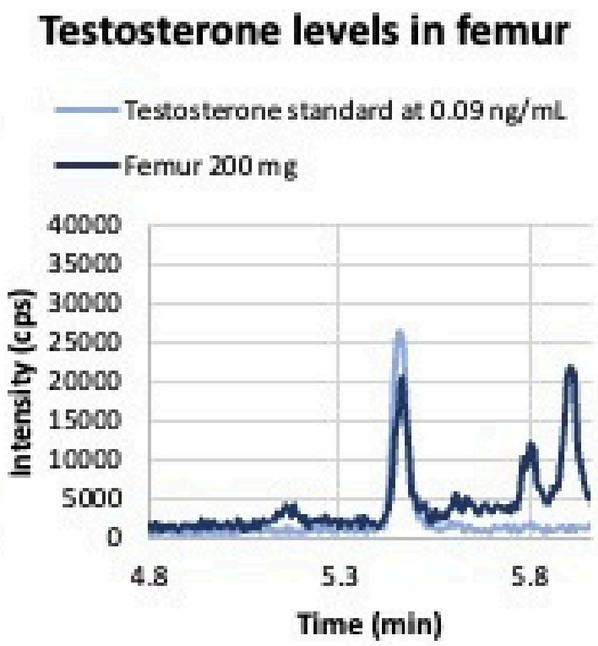
RESULTS

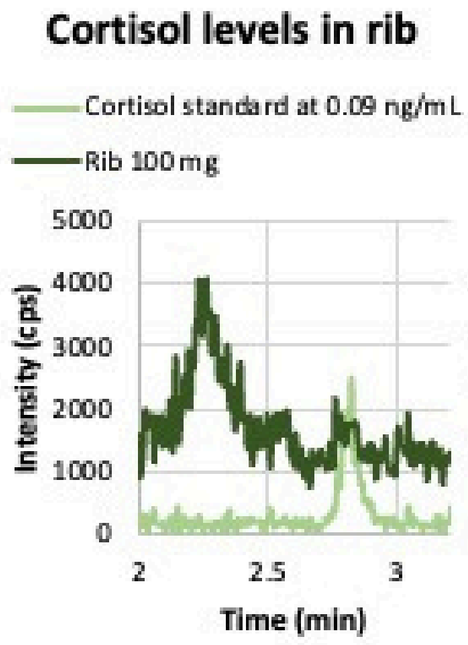
1. ~~HCl demineralization rib (200mg)~~
2. ~~EDTA deminerlizatoin rib (200mg)~~
3. ~~Ground bone rib (200mg)~~
4. MeOH extraction femur cortical (200mg)
5. MeOH extraction femur cortical (300-400mg)
6. MeOH rib (50, 100 mg)
7. MeOH femur cortical (50, 100, 200mg)

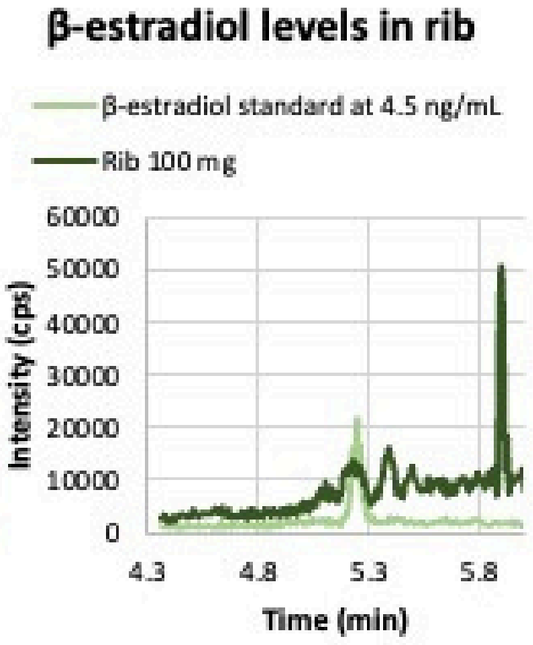

Cortisol levels in femur
Cortisol standard at 0.09 ng/mL
Femur 200 mg
Intensity (cps)
5000
4000
3000
2000
1000
0
1.65
2.65
Time (min)

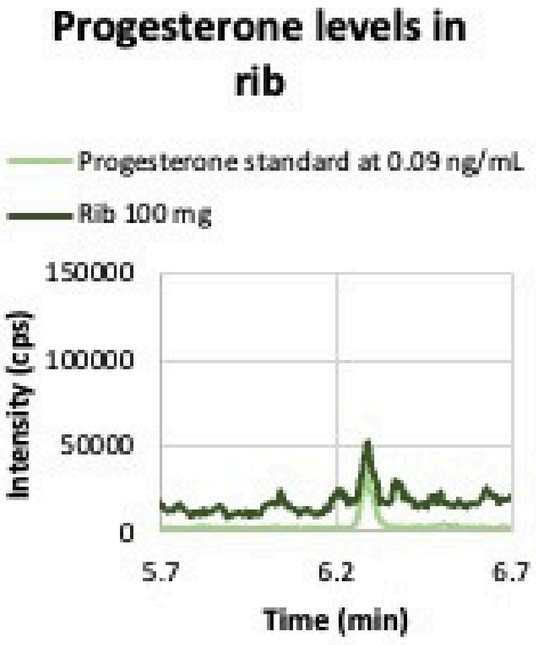

β-estradiol levels in femur
β-estradiol standard at 4.5 ng/mL
Femur 200 mg
Intensity (cps)
25000
20000
15000
10000
5000
0
4.3
4.8
5.3
5.8
Time (min)

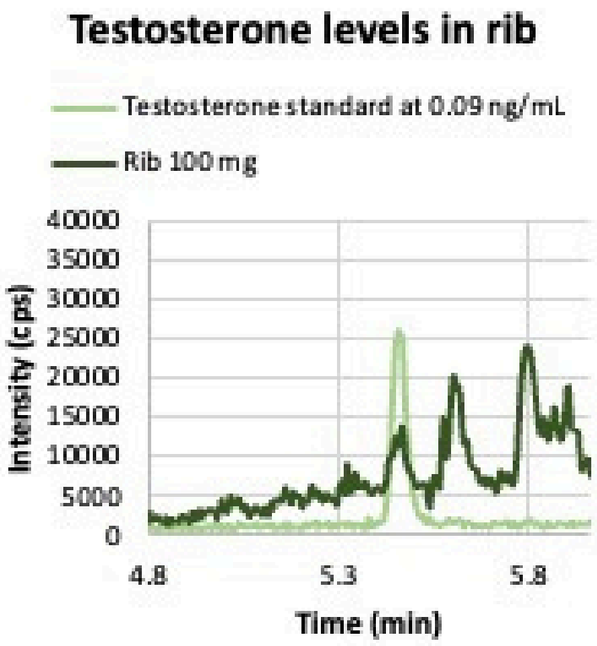

Progesterone levels in femur
Progesterone standard at 0.09 ng/mL
Femur 200 mg
Intensity (cps)
150000
100000
50000
0
5.7
6.2
6.7
Time (min)

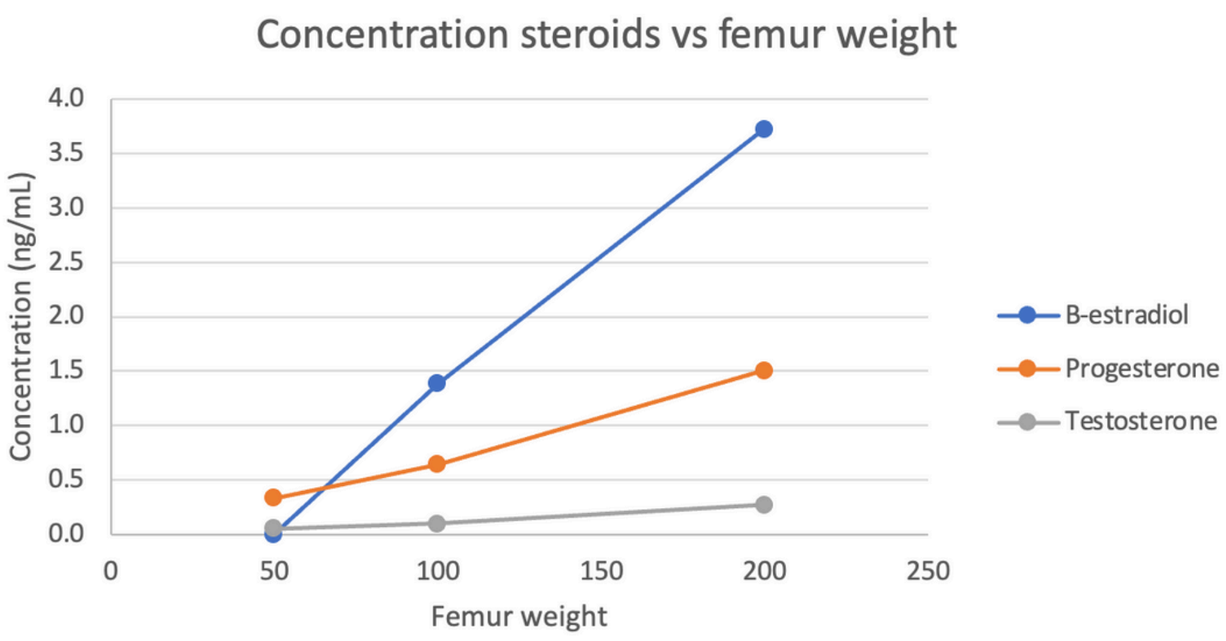

Testosterone levels in femur
Testosterone standard at 0.09 ng/mL
Femur 200 mg
Intensity (cps)
40000
35000
30000
25000
20000
15000
10000
5000
0
4.8
5.3
5.8
Time (min)

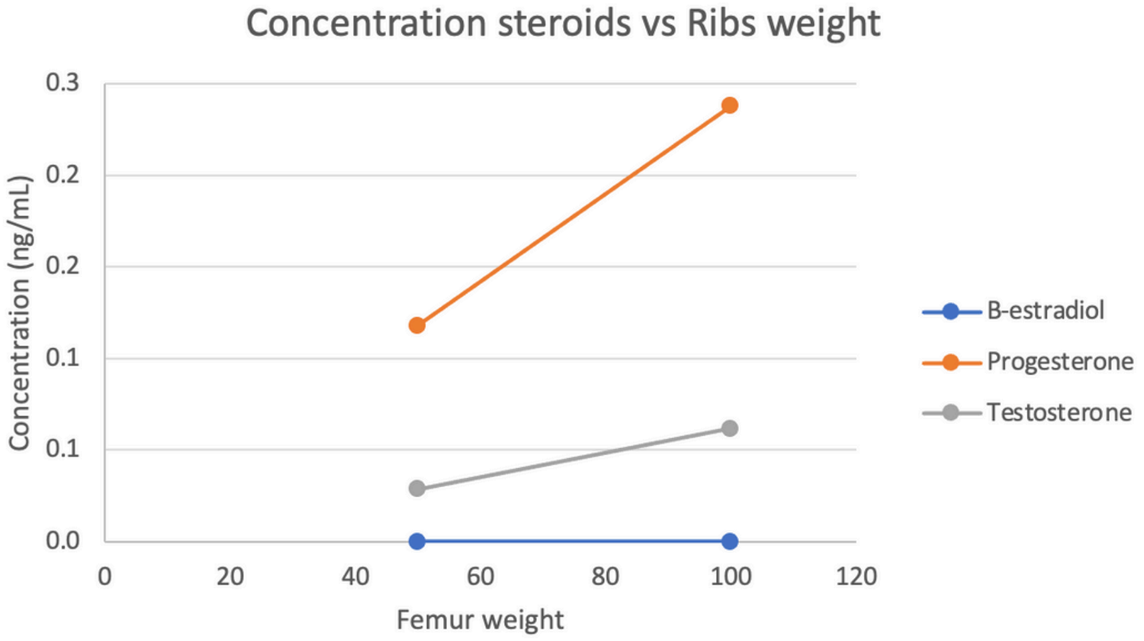

Cortisol levels in rib
Cortisol standard at 0.09 ng/mL
Rib 100 mg
Intensity (cps)
5000
4000
3000
2000
1000
0
2
2.5
3
Time (min)


β-estradiol levels in rib
β-estradiol standard at 4.5 ng/mL
Rib 100 mg
Intensity (cps)
60000
50000
40000
30000
20000
10000
0
4.3
4.8
5.3
5.8
Time (min)


Progesterone levels in rib
Progesterone standard at 0.09 ng/mL
Rib 100 mg
Intensity (cps)
150000
100000
50000
0
5.7
6.2
6.7
Time (min)


Testosterone levels in rib
Testosterone standard at 0.09 ng/mL
Rib 100 mg
Intensity (cps)
40000
35000
30000
25000
20000
15000
10000
5000
0
4.8
5.3
5.8
Time (min)


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!

S.A.SCRADER@ARCH.LEIDENUNIV.NL
@DRSARAHSCHRADER

WE WOULD LIKE TO THANK MARTIJN DEFILET, GEMEENTE ARNHEM, AND THE ARNHEM C FOR ALLOWING US TO ANALYZE THESE REMAINS.

THIS PRESENTATION IS PART OF THE PROJECT EVOLUTION OF STRESS (406.XS.01.118) OF THE RESEARCH PROGRAMME OPEN COMPETITION, WHICH IS FINANCED BY THE DUTCH RESEARCH COUNCIL (NWO)