Male FHM Urine-Based Metabolomics for Assessing Impacts of Chemical Stressors

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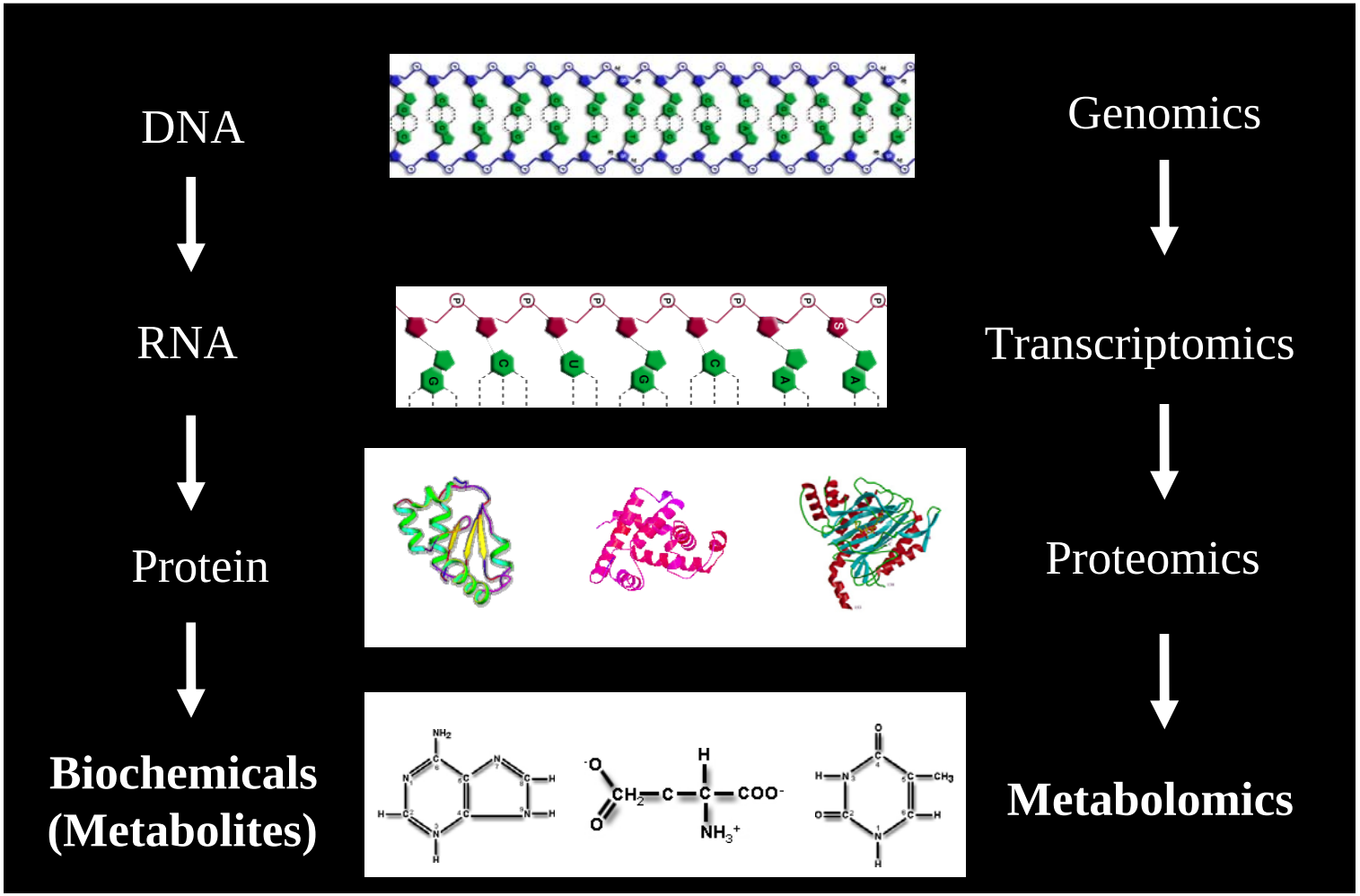
The Central Dogma



Metabolon, Inc., 2004

What is Metabolomics?

- Metabolomics involves the rapid, **high throughput characterization** of the small molecule endogenous metabolites found in an organism.

Where to Sample?

- Tissues - allows more restricted/focused analysis
  - Liver
  - Brain
  - Gonad
  - Kidney
  - Etc.

- Biofluids – allows more systemic analysis
  - Blood
  - Urine
  - Etc.

Advantages of Using Urine for Metabolomics Studies

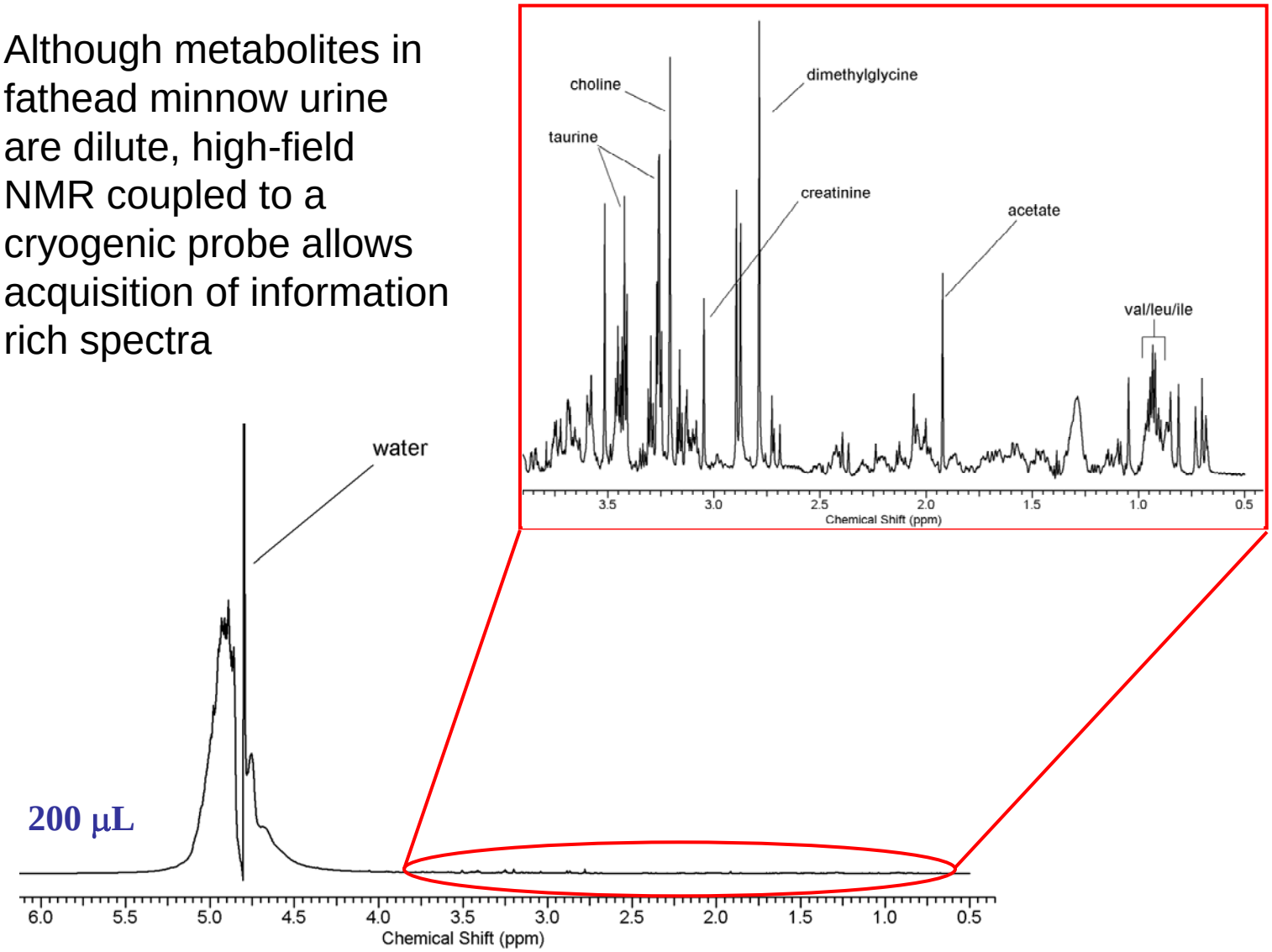
- Endpoint indicative of an organism's integrated metabolic function
  - can be useful for determining multi-organ responses to disease or toxicity
- Requires minimal sample preparation
- Can be collected without sacrificing the animal
  - possible to collect multiple samples from a single individual over time

*Disadvantages of Using Urine for Metabolomics Studies with **Fish***

- Difficult to obtain
  - Requires gentle pressure on abdomen and collection from cloaca *via* non-heparinized capillary tube
- Contamination of sample can obscure toxicity response
  - sperm
  - feces
- Dilute
  - Freshwater fish constantly excrete urine due to osmotic pressure (the fish is hypertonic).
- Relatively small volumes obtainable from a single fish.
  - Collected volumes generally range from 0 to 30 μL

Fathead Minnow Urine Spectra

Although metabolites in fathead minnow urine are dilute, high-field NMR coupled to a cryogenic probe allows acquisition of information rich spectra



Assignment of ^{1}H and ^{13}C NMR-detectable Metabolites in Male FHM Urine

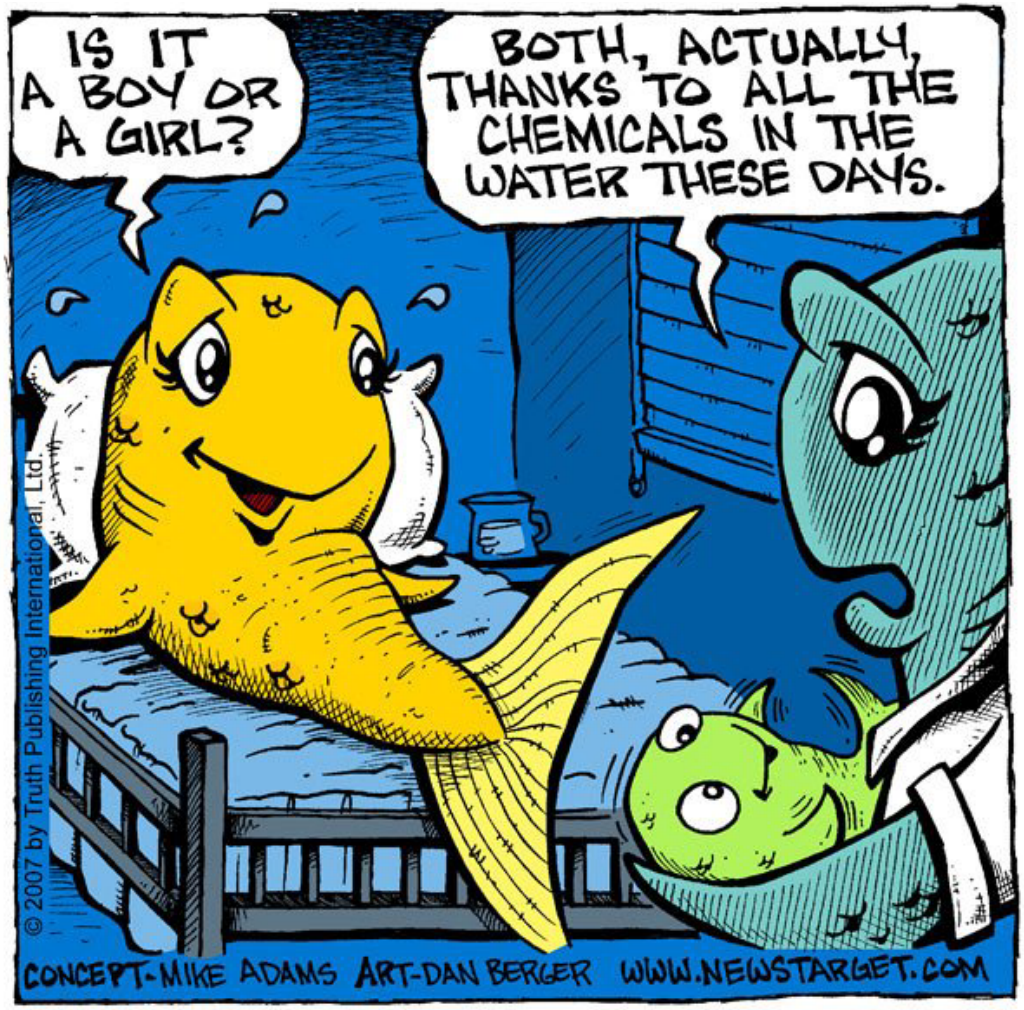
^{1}H and ^{13}C NMR chemical shift assignments for metabolites observed in urine from unexposed male fathead minnows[a]

Metabolite	^{1}H Chemical shift(s) (ppm)	^{13}C Chemical shift(s) (ppm)
Unidentified fatty acids	0.87, 1.30, 1.56, 2.22	24.63, 29.97
Unidentified lipid resonances	0.86, 1.28	
Unidentified monounsaturated lipid (MUL)	0.90, 1.47, 2.20, 5.95, 6.80	15.57, 23.95, 36.39, 125.54, 149.70, 171.98
Valine	0.96, 1.04	
Lactate	1.32	
Alanine	1.46	
Acetate	1.91	184.18
Proline	2.11, 2.25, 3.26, 3.37, 4.17	
Dimethylamine	2.70	37.60
Dimethylglycine	2.78	47.20
Trimethylamine	2.89	47.43
Creatine	3.04, 3.96	59.33, 172.02,
Creatinine	3.05, 4.06	32.97, 172.26, 191.66
Choline	3.20, 3.50	56.05, 62.19
Taurine	3.25, 3.42	38.16, 50.06
Trimethylamine-*N*-oxide	3.26	60.86
Betaine	3.26, 3.89	56.21, 68.76, 171.94
Tryptophan	3.30, 3.46	
Hippurate	3.95, 7.54, 7.63, 7.78	
Phenylalanine	7.33, 7.35, 7.42	
Formate	8.44	

- A wide variety of metabolites can be detected.
- Various classes can be used to determine effects on: energy metabolism, amino acid anabolism/catabolism, osmolytes, etc.
- Currently developing MS-based methods to increase number and variety of detectable metabolites.

Male FHM Urine Metabolite Profiling for Assessing Impacts of EDCs

Phenotypic Impacts of EDCs



Difficult to Assess Exposure to Anti-androgens

- Good fish-based whole-animal assays for many EDC modes of action
  - Vtg induction in males exposed to estrogens
  - Male secondary sex characteristic induction in females exposed to androgens
- This is not generally true for anti-androgens
  - Responses observed for flutamide and vinclozolin are subtle and not mode of action specific

Indirect Methods of Detection

- Anti-androgen MOA is often determined through indirect methods.

- Inhibition of androgen effects
  - Co-exposure with an anti-androgen and an androgen
  - look for blockage of the androgen effect.

Potential of Male FHM Urine for Directly Detecting an Anti-androgen?

NMR analysis of male fathead minnow urinary metabolites: A potential approach for studying impacts of chemical exposures☆

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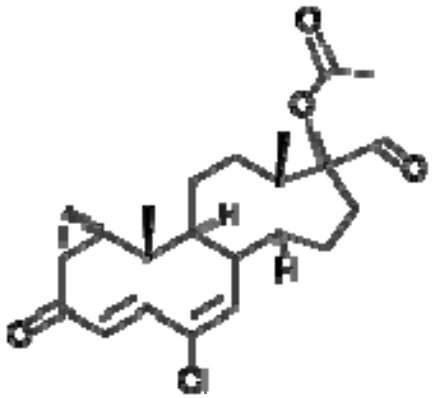
- Proof of concept study
  - Developed method
  - Identified metabolites
  - Determined effects in males due to vinclozolin (VZ) exposure
- Since VZ is an anti-androgen we hypothesized that urine might be useful for direct detection of an anti-androgenic effect
  - But it was just a small set of samples (subset from an exposure)

Male FHM Exposure to Cyproterone Acetate

- Cyproterone Acetate (CA) – a model mammalian androgen receptor (AR) antagonist
  - pharmaceutical often administered for the treatment of prostate cancer.

- Potentially useful for assessing the ability of FHM urine metabolite profiling to directly characterize the impact(s) of exposure to an Androgen Receptor (AR) antagonist



Exposure Scenario

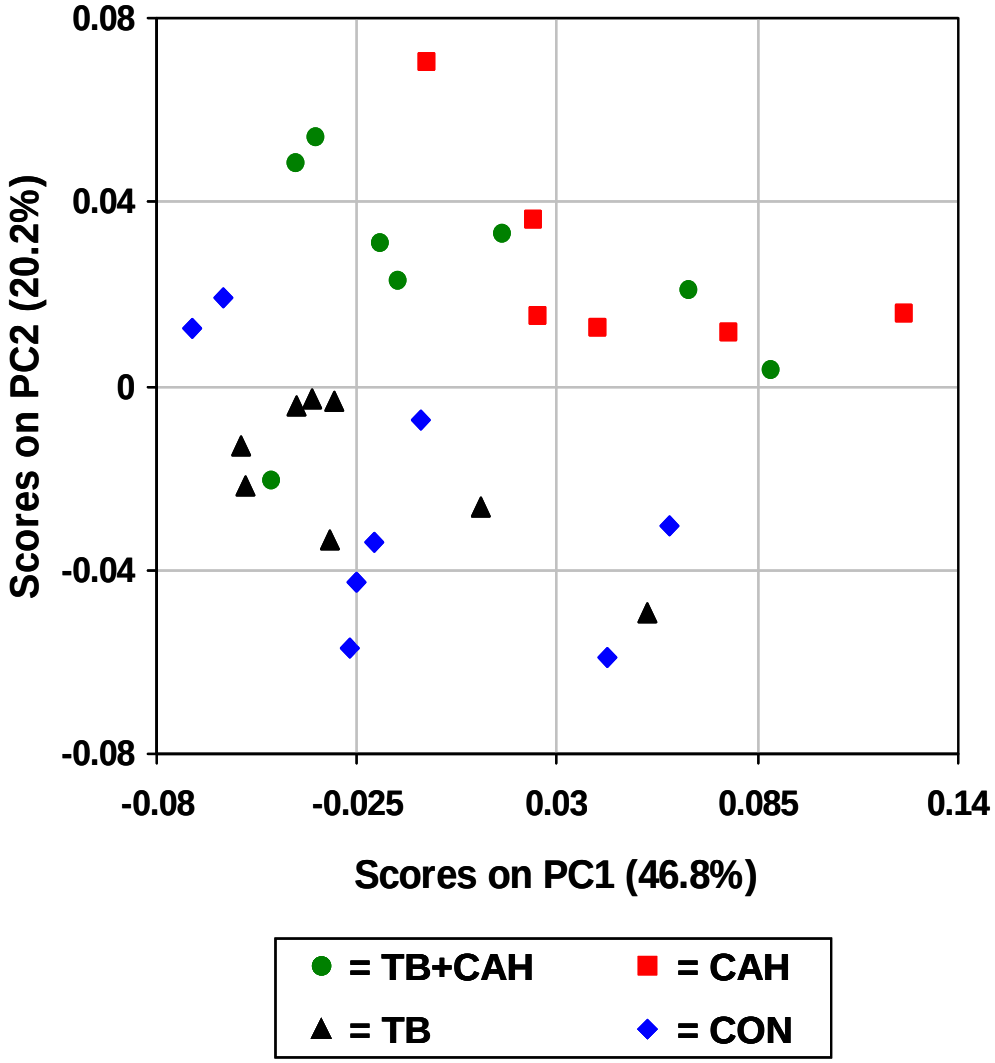
- Six treatments:
  - Control
  - 200 μg/L CA (CAH)
  - 20 μg/L CA (CAL)
  - 200 μg/L CA + 500 ng/L TB
  - 20 μg/L CA + 500 ng/L TB
  - 500 ng/L TB

← to test specificity of CA for the FHM androgen receptor (AR)

- Urine samples collected after 14 days of exposure

- Urine collected for most males (about 96%)

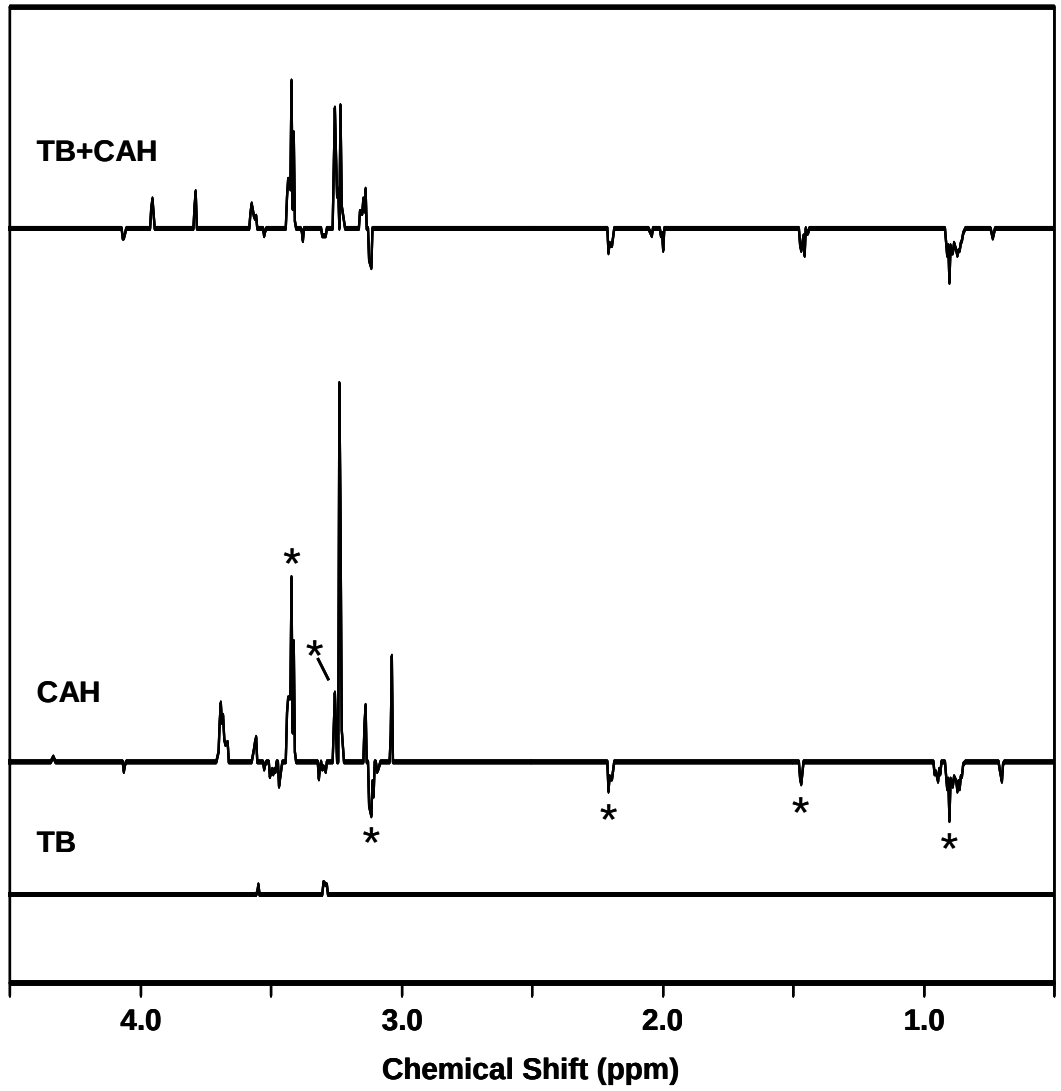
High Concentration of Cyproterone Acetate (CAH) Produces Large Impact on Urinary Metabolite Profile



- TB alone has little effect.
- CAH produces a large response.
- TB + CAH suggests possible blocking of CAH effect.

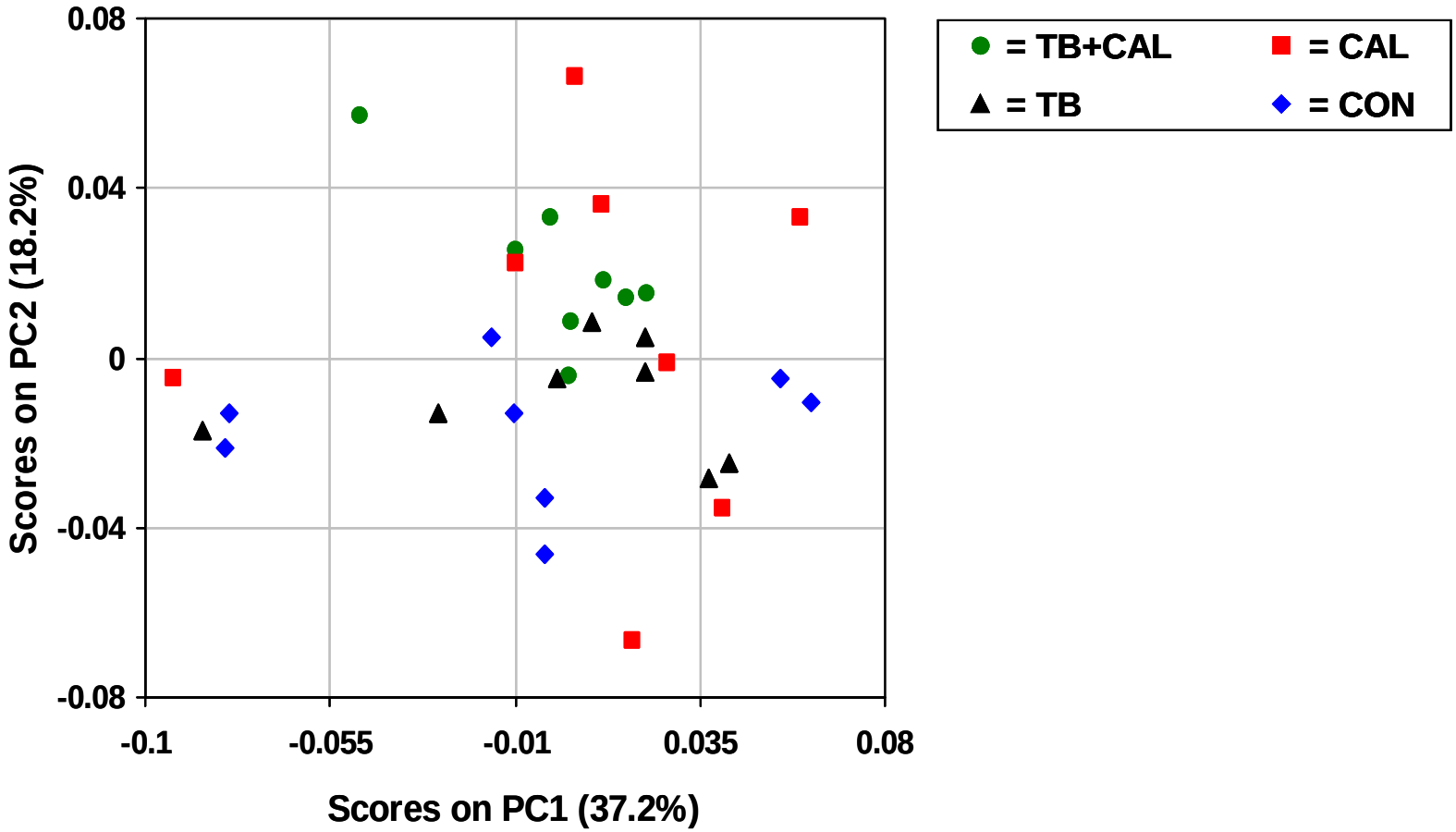
t-test Filtered Average Difference Spectra

further confirms that the presence of TRB REDUCES effect of CA-HI in males



Impact of Exposure to Low Concentration of Cyproterone Acetate (CAH)

suggests that presence of TRB INCREASES effect of CA-LO in males



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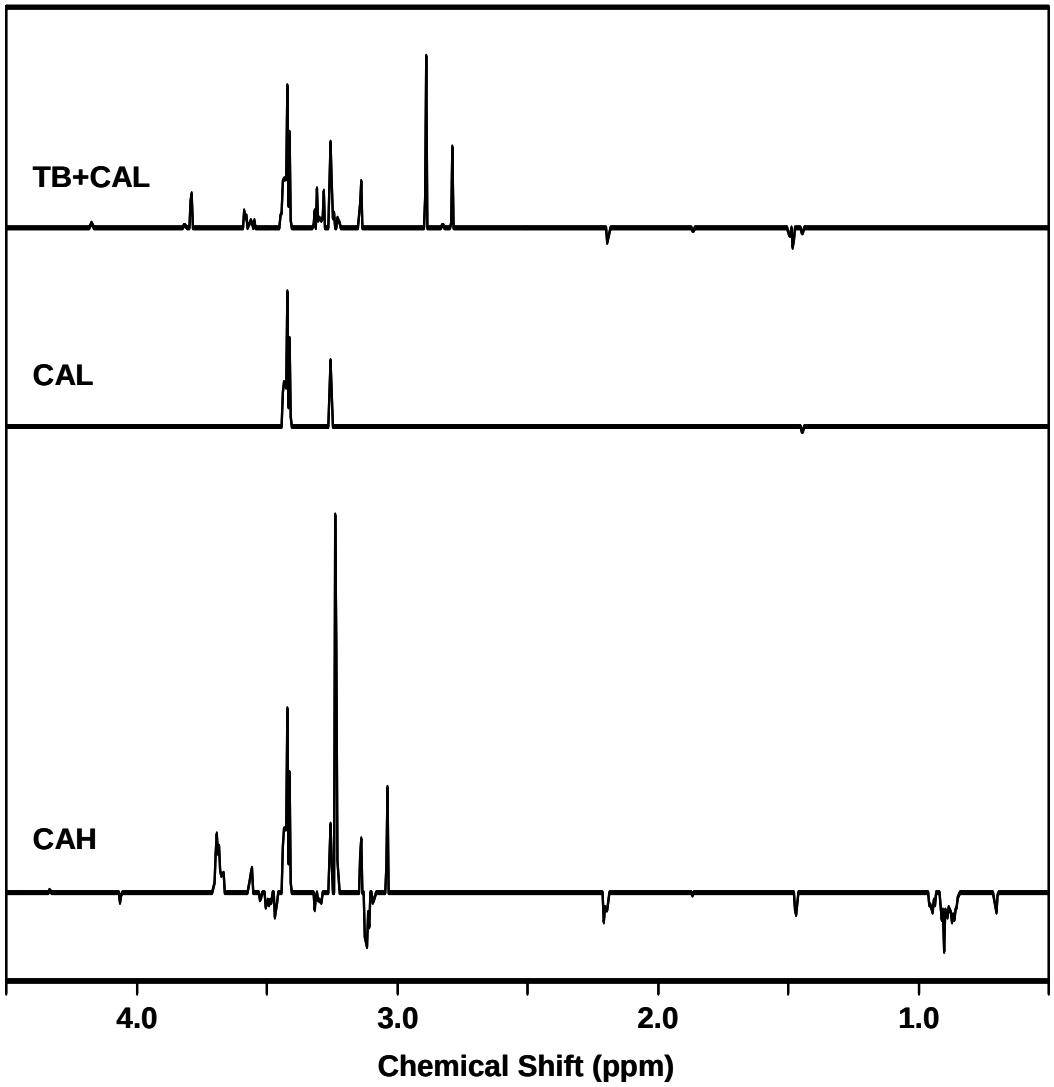

Next Steps

- Identifying metabolite changes induced by exposure to cyproterone acetate alone as well its co-exposure with 17β-trenbolone
- Search for biomarkers of anti-androgen exposure in urine
- Conduct exposures with other anti-androgens to test ability to detect impact directly with male FHM urine

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