Gut Bacterial Conversion of Endogenous Corticoids into GALFs

HARVARD CHEMICAL BIOLOGY PHD PROGRAM

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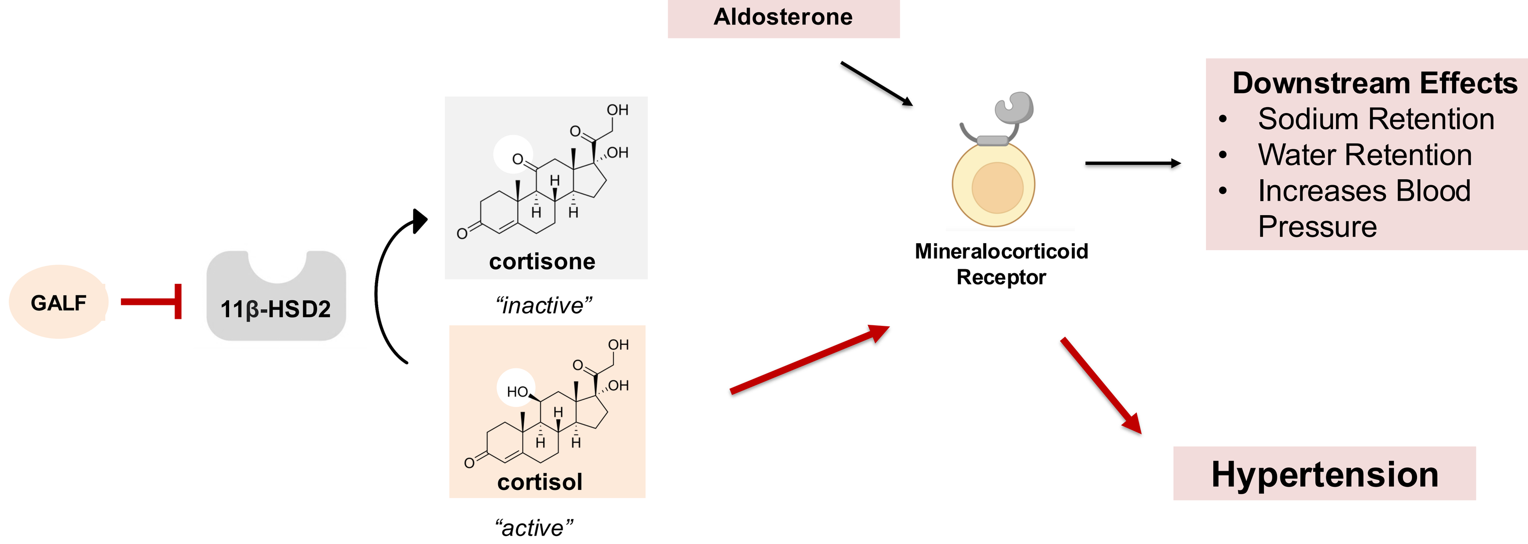
Background

Glycyrrhetinic Acid-Like Factors (GALFs) Promote Hypertension

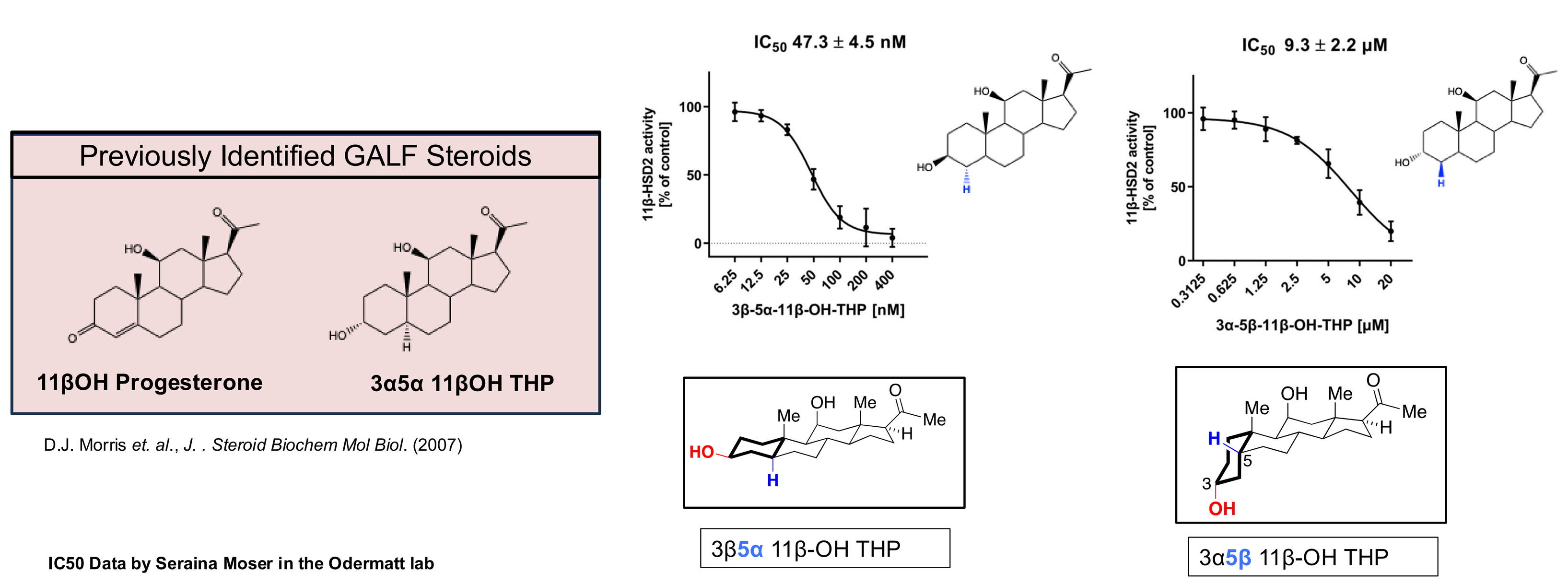
Downstream Effects
- Electrolyte depletion
- High blood pressure
- Cardiac arrest
- In rare cases death

Glycyrrhetinic acid, a compound found in black licorice promotes hypertension when overconsumed.



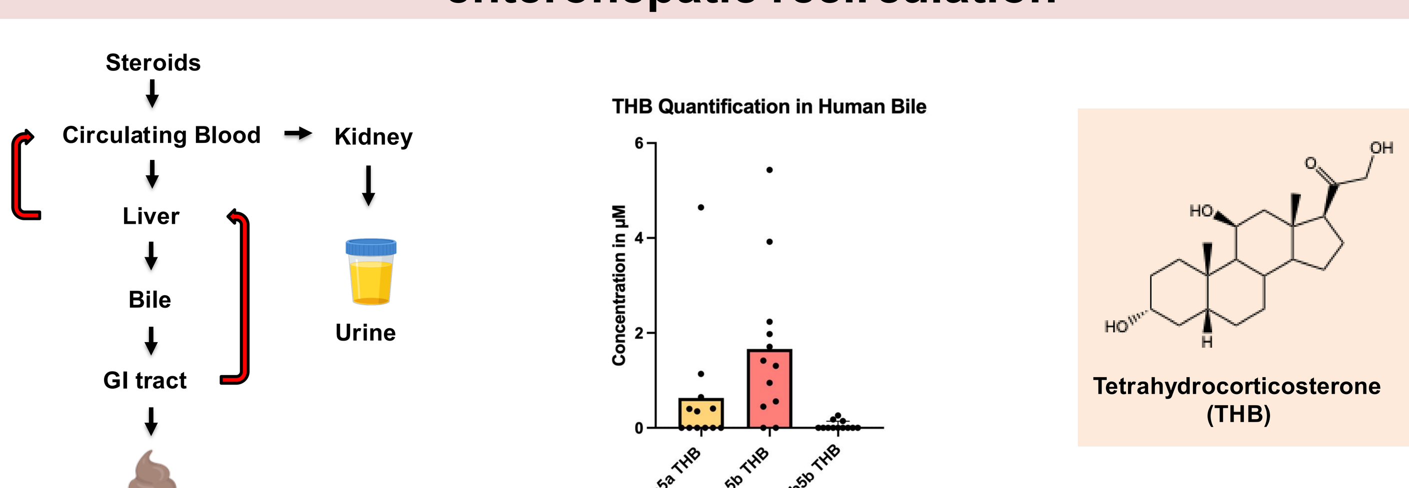
Downstream Effects
- Sodium Retention
- Water Retention
- Increases Blood Pressure

The body maintains blood pressure homeostasis through release of aldosterone when blood pressure is low. Aldosterone activates the mineralocorticoid receptor (MR), the downstream effects of which are increasing blood pressure to homeostatic levels. Cortisol has the same binding affinity to MR but is deactivated through the action of the enzyme 11β-HSD2. GALFs block 11β-HSD2, leading to increased activation of MR through cortisol and potential hypertension.



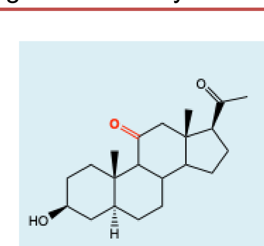
David Morris' group identified endogenous steroid metabolites that inhibit 11β-HSD2 and hypothesized that gut bacteria could convert endogenous steroids into GALFs.

Steroid hormone metabolites can undergo enterohepatic recirculation



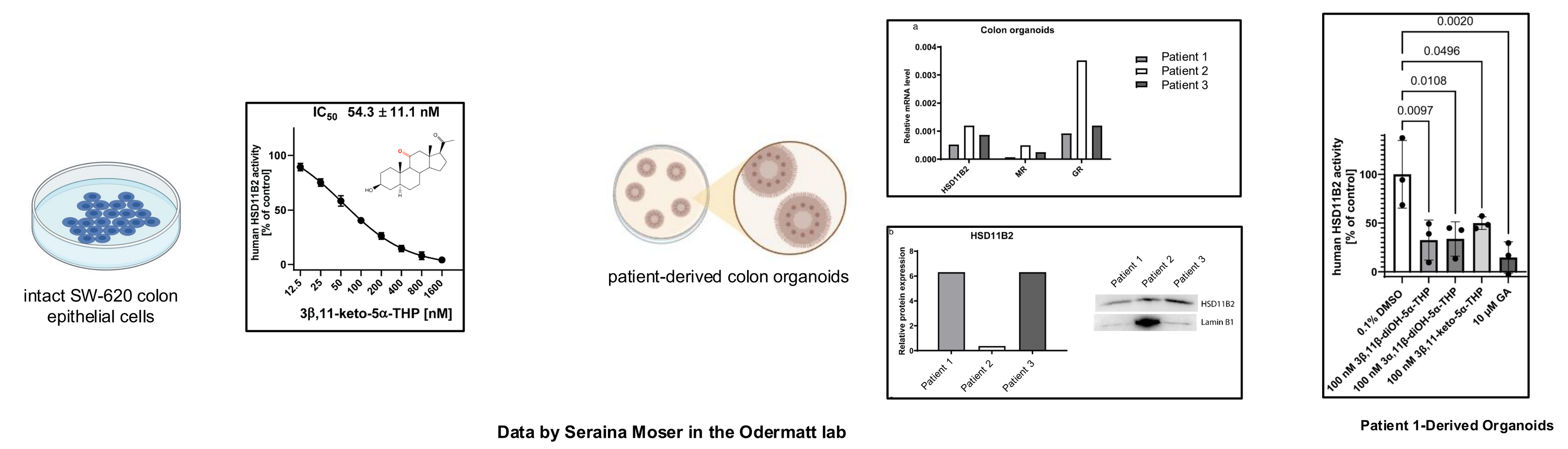
THB isomers were found in μM conc. in human bile samples from Primary Sclerosing Cholangitis and Biliary Stricture patients.

Isomer	IC50	# of Samples Found	Analysis	Spike-In With Standard?
3β5α 11βOH THP	50nM	0/16	Full Scan/tMS2	yes
3α5α 11βOH THP	120nM	0/16	Full Scan/tMS2	yes
3α5α 11keto THP	??	0/16	Full Scan/tMS2	yes
3β5α 11keto THP	**150nM**	**10/16**	**Full Scan/tMS2**	**yes**



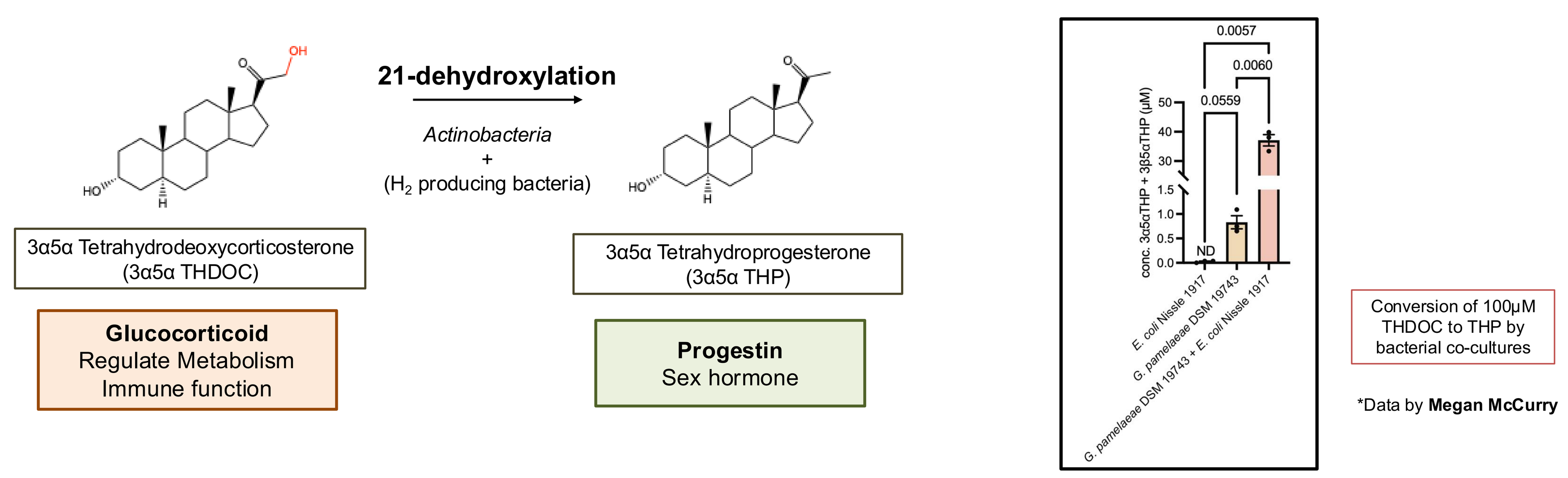
Out of 16 human fecal samples analyzed for the presence of GALFs, 10 had peaks for 3β5α 11keto THP. The peak was verified through targeted MS2 and spike-in with a standard.

3β5α 11keto THP has low IC50 towards human 11β-HSD2



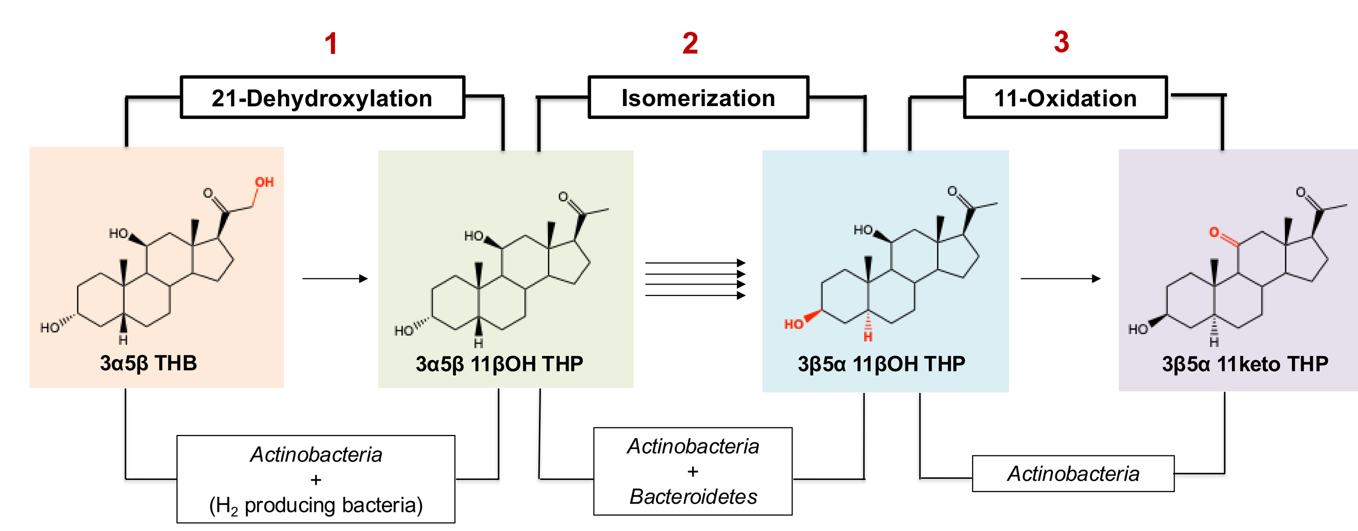
On the left, the IC50 of 3β5α 11keto THP is 54.3 against endogenous 11β-HSD2 in a human colon epithelial cell line. On the right, 100 nM 3β5α 11keto THP reduces 11β-HSD2 activity in patient-derived colon organoids.by half.

The gut microbiome performs 21-dehydroxylation of steroids



Previous work in the lab has shown that gut microbes from the *Eggerthella* and *Gordonibacter* genus 21-dehydroxylate steroids converting them from glucocorticoids to progestins, a new steroid class.

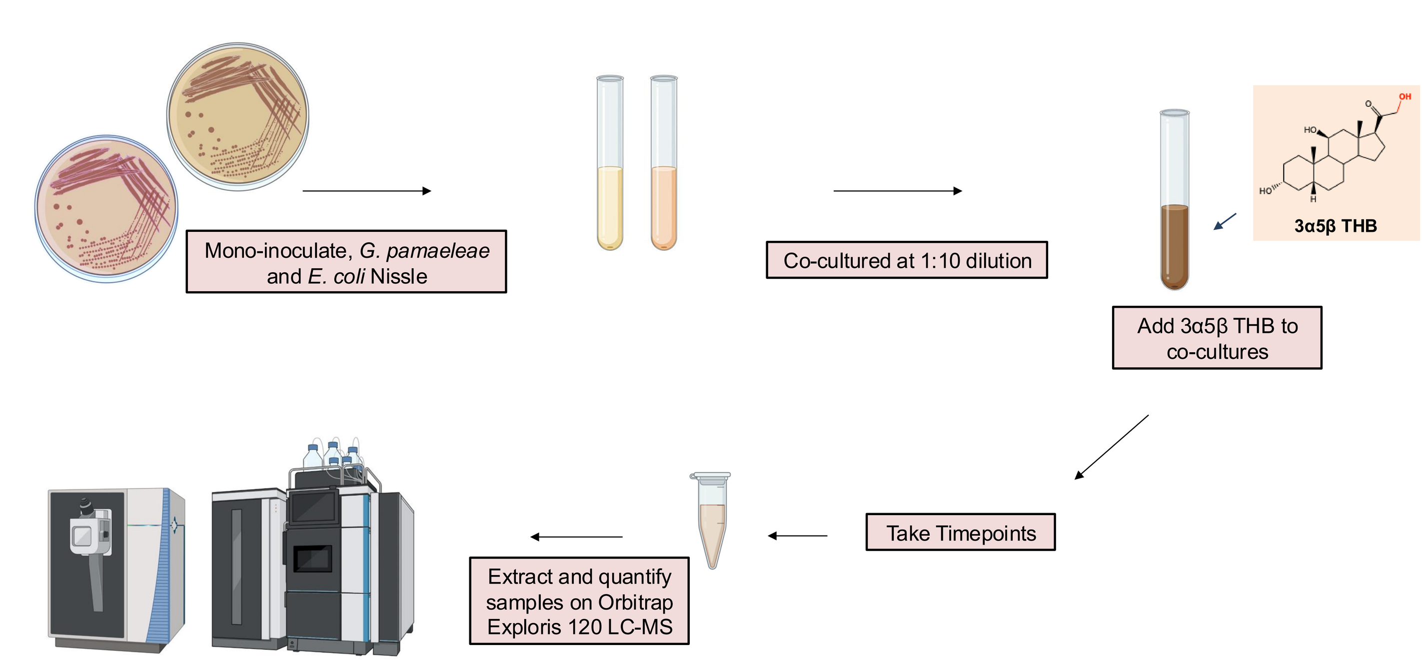
Corticoid to GALF Bacterial Production Pathway



Goal: Elucidate bacterial production of the GALF 3β5α 11keto THP from 3α5β THB.

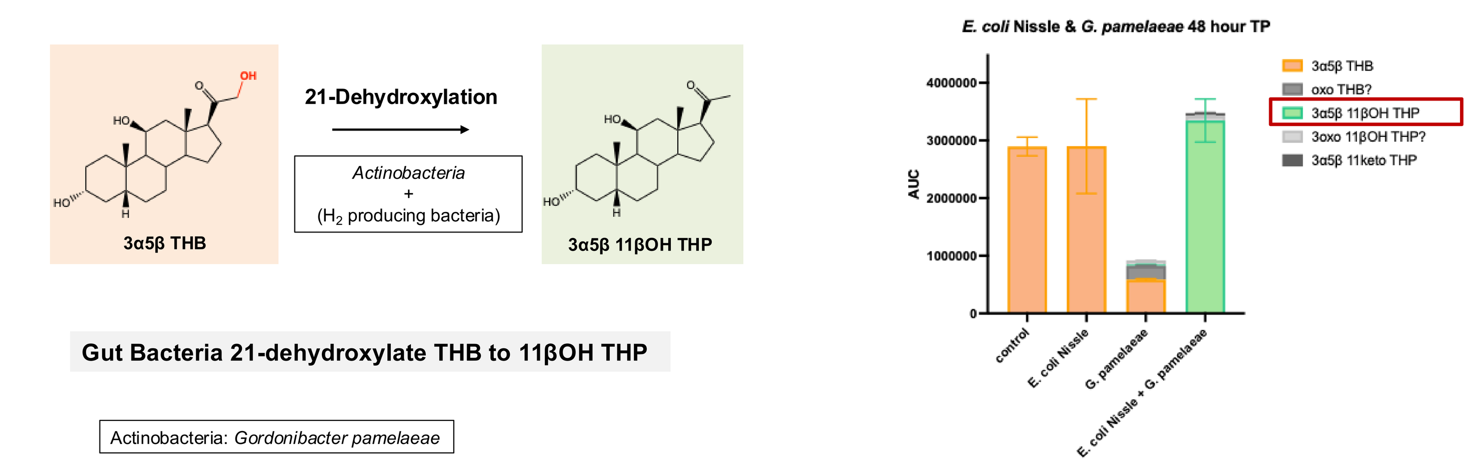
Results

General workflow of co-culture experiments



Workflow of the first experiment co-culturing *G. pamelaeae* and *E. coli* Nissle. Other experiments shown have similar workflows.

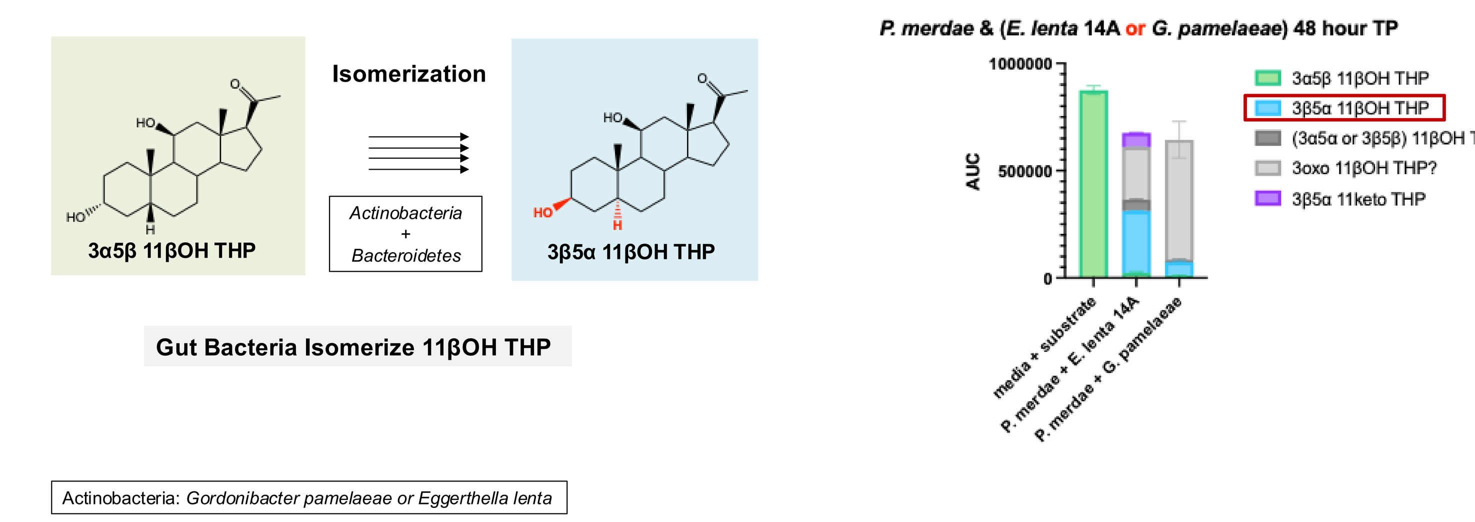
21-dehydroxylation of THB to 3α5β 11βOH THP



Gut Bacteria 21-dehydroxylate THB to 11βOH THP

Actinobacteria: *Gordonibacter pamelaeae*

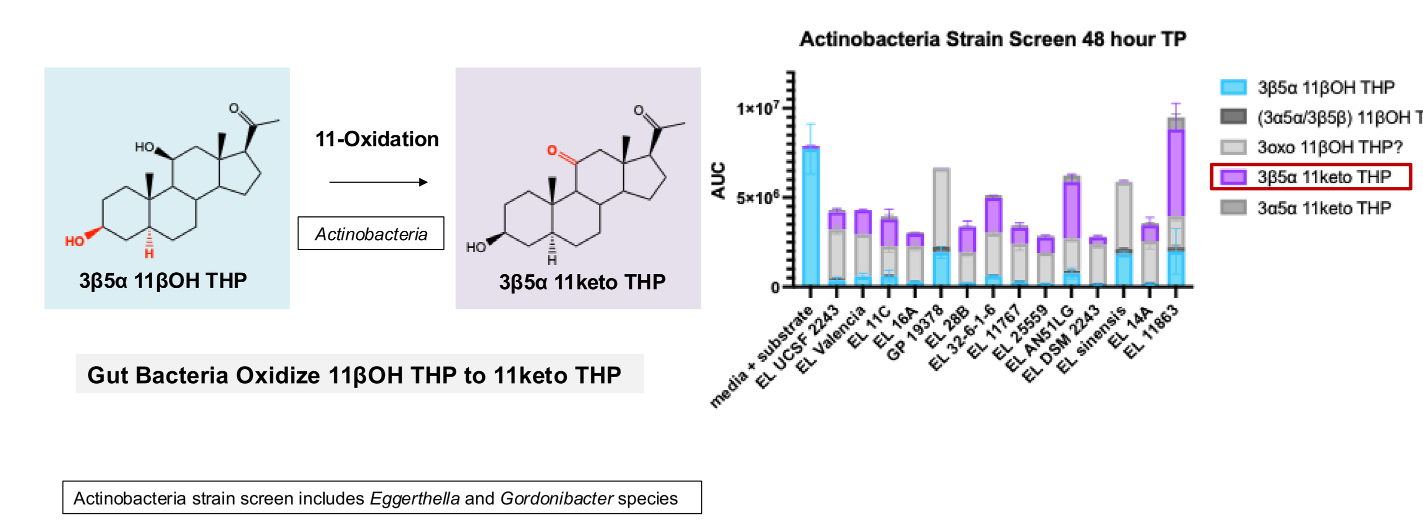
Isomerization of 3α5β 11βOH THP to 3β5α 11βOH THP



Gut Bacteria Isomerize 11βOH THP

Actinobacteria: *Gordonibacter pamelaeae or Eggerthella lenta*

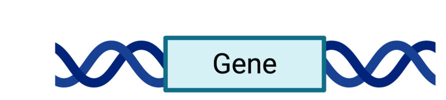
11-Oxidation of 3β5α 11βOH THP to 3β5α 11keto THP



Gut Bacteria Oxidize 11βOH THP to 11keto THP

Actinobacteria strain screen includes *Eggerthella* and *Gordonibacter* species

Ongoing and Future Work

Quantification of GALFs from human hypertension patient samples	11β-HSDH gene identification
	
Quantify levels of bacterial GALFs in fecal samples from patients with hypertension from collaboration with Mingyang Song in HSPH	Determine the gene responsible for 11-oxidation in Actinobacteria

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