University of BRISTOL

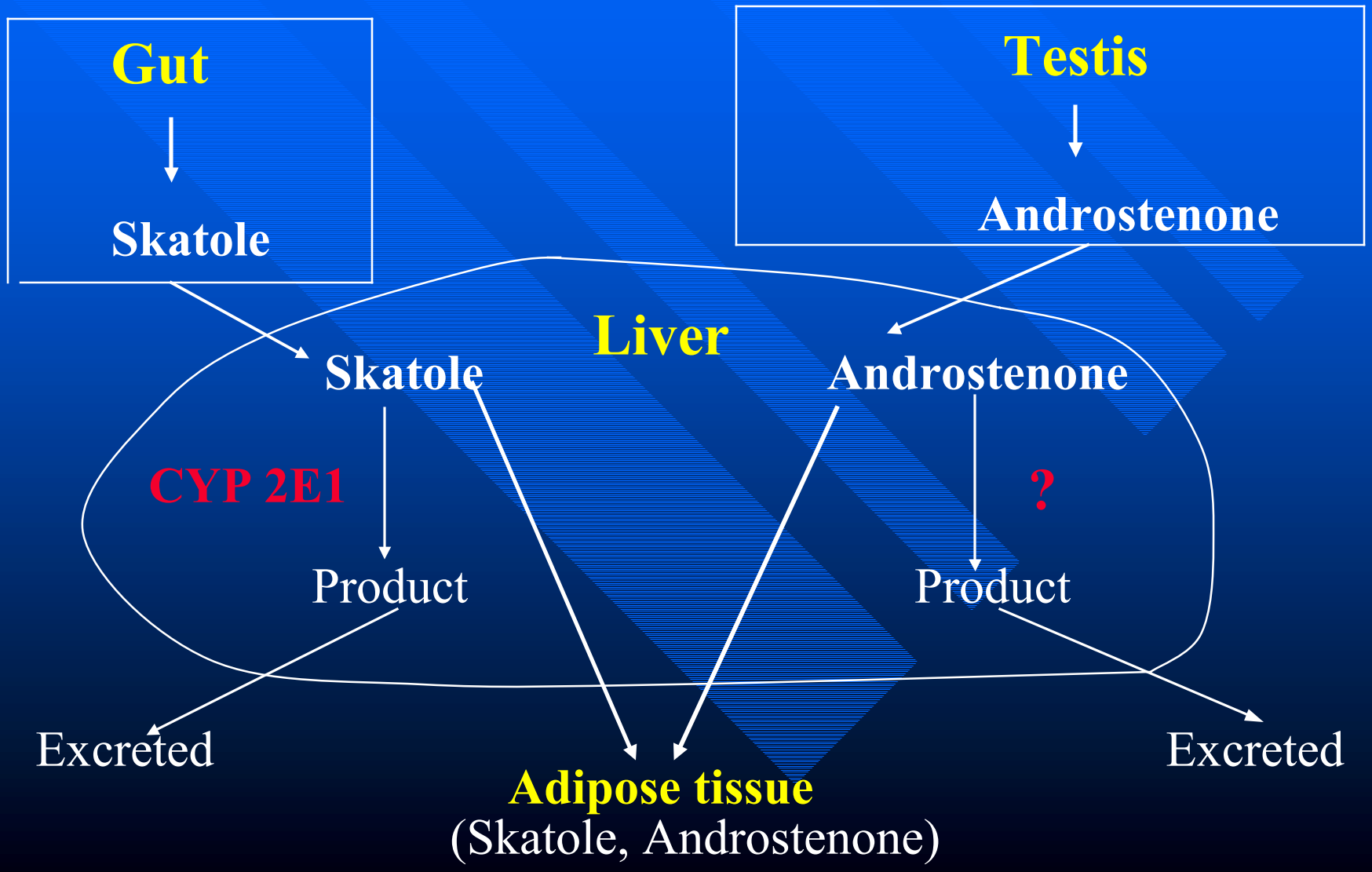
Inhibition of CYP2E1 expression by androstenone: relation to boar taint

O.Doran, J.D.McGivan, F.M.Whittington,
W.S.Tambyrajah J.D.Wood

Boar taint

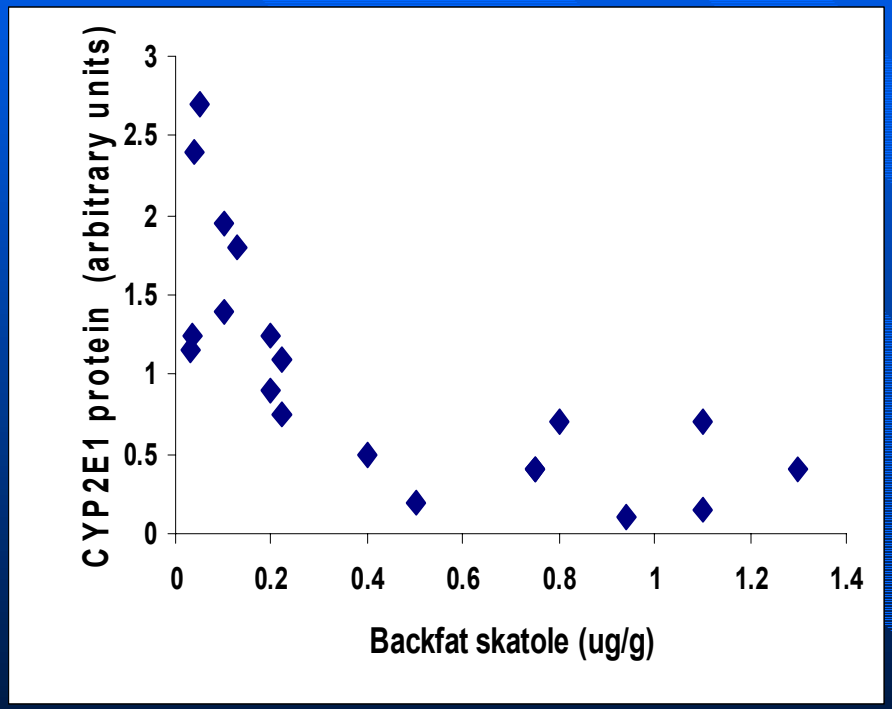
- Boar taint is an offensive odour in the meat of 5-10% of uncastrated male pigs
- Is due to excessive accumulation in adipose tissue of the natural products skatole and androstenone
- Can be eliminated by castration
- An alternative to castration could be a genetic test

Skatole and androstenone synthesis and degradation

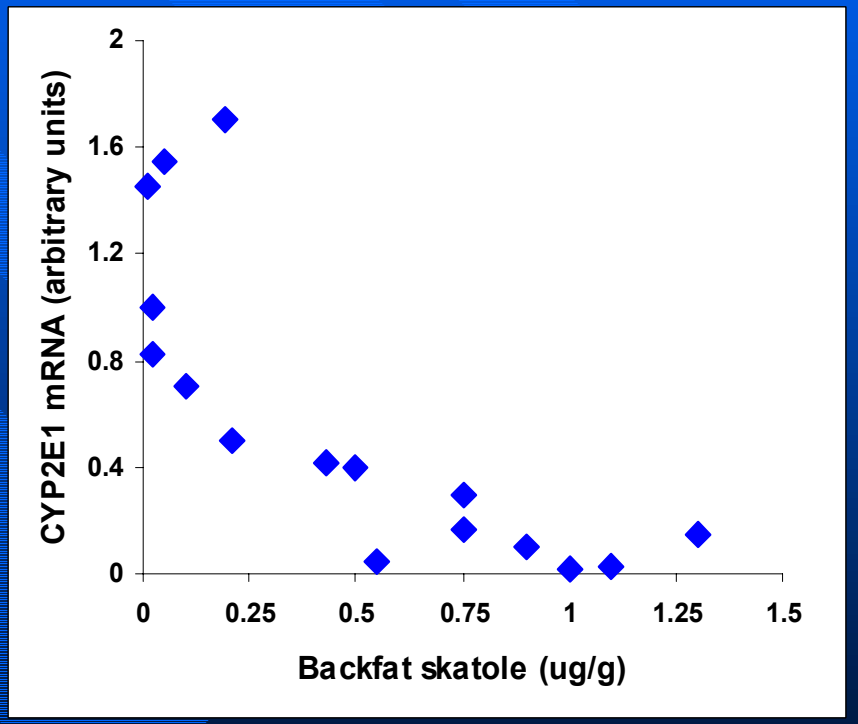


CYP2E1 expression in pig liver

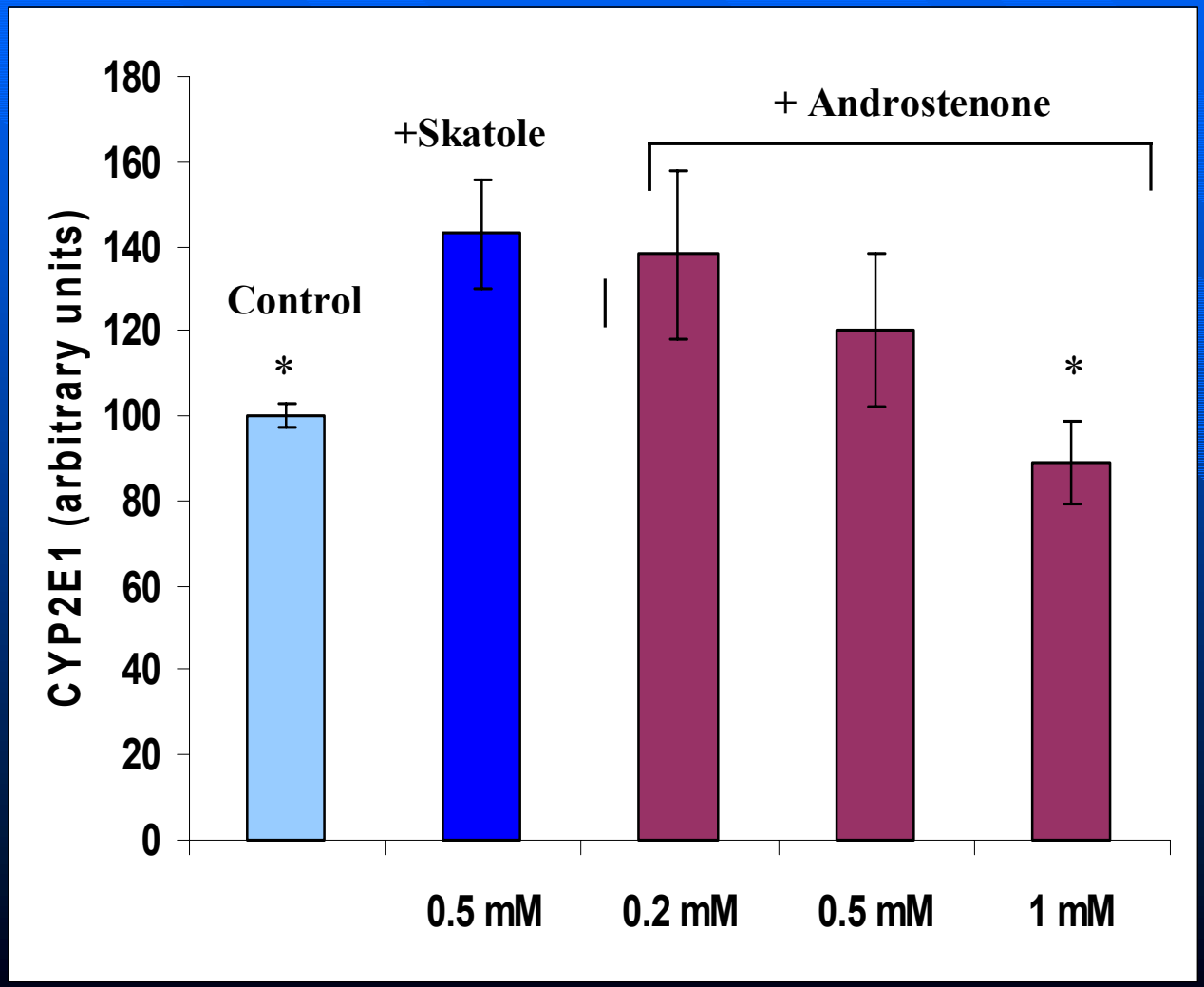
CYP2E1 protein



CYP2E1 mRNA



Effect of androstenone CYP2E1 protein expression in cell culture



Objectives

- To investigate the molecular mechanism regulating CYP2E1 expression
- To investigate the mechanism regulating androstenone deposition

Investigation of the molecular mechanism regulating CYP2E1 expression

- To sequence the promoter of the pig CYP2E1 gene
- To identify regulatory elements in the pig CYP2E1 promoter
- To identify transcription factor(s) binding to these regulatory elements
- To investigate effect of androstenone on binding of the transcription factors to CYP2E1 promoter

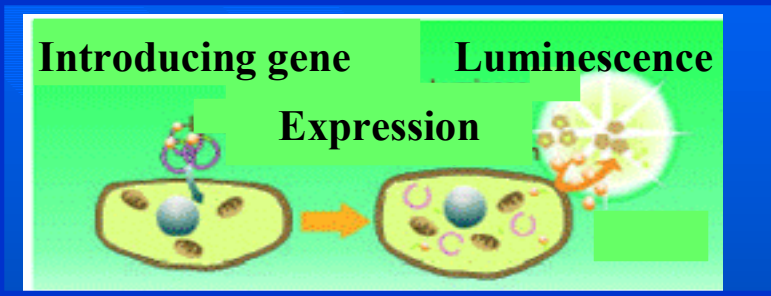
CYP2E1 Promoter

```
-850                                                  CCCCA GAAACAACCT

-800 AACAGAAAGG TGAATGTAAA TAGTTTGGAG CTCTTATTTT AAATGAGAAT

-750 GTCCACACAC ATTAGCACAG ATTTAAACAA ACACAGTTAA AATACTAATT

-700 TTTTTTTTAG AATTTGACAA AATGACTCTA AACAGTAATT ATCCCTTAAT

-650 TTTCTACAGT AAAAATATAC CCTTTTTTGG TAGTAATCAG AGATGAACTT

-600 TTTTGAAATT TGTCAACTCT TTTCCTTTCT CTTTTCCTCC CCCACTGAAT

-550 TTGCCAGTTG ATTTCCCAAA GTGGAGTGAA ATTCAGATAC TGAATTTCCC

-500 TTCTCTGGCC CATGAGGCTG GCTGCTGATG ACTCAGTACC ACTGGGGTTG

-450 CTCAGACAGA CCTGCTCGGA GGCTGAGAGT TGCACCAGGA GATGGAGCAA

-400 GACGGTCGGC ACATCATTGA TGTCGCCTTA CATAAATCCT ACCCCAAACA

-350 AACCCATGTA AATATGACCT TCTTGTCCAA CCAAGGTAAA GGAGAGGACA

-300 GTTCCCCACC CTATGTTCTG ACCTCTGGGT TGGTGGAGCT AAACTGGATG

-250 ACATGTTTTA CTGACATTGG TGCAGGTGTC AGCAGCCAGT GTTGGCAGAG

-200 CCCAGGCTAG AGGAAGTGAG TGTCTGGATG GAGTTCTAAG GGGTAACCGC

                HNF-4                                 HNF-1
-150 CTCAGGGATC AGCCTTTGAA CTGATAGCCA ACAGCAGCTA ATAATAAACC

-100 TATATCTTGG GCTGGAGGAA AAGGAAGGTG GCATTGGTTG GCTGGTCACC

                      TATA box
-50  CTCCTTCTCA AGGATGCATT ATAAAAGGCT GCCTCTCCAC AGGAGCATCT

-0   CCACACATTG AAAGATCCCC TGAAGGAGCC ATG
```

Sequenced by T.Skinner, O.Doran, J.McGivan, A.Archibald, C.Haley, GenBank Accession No AJ 697882.

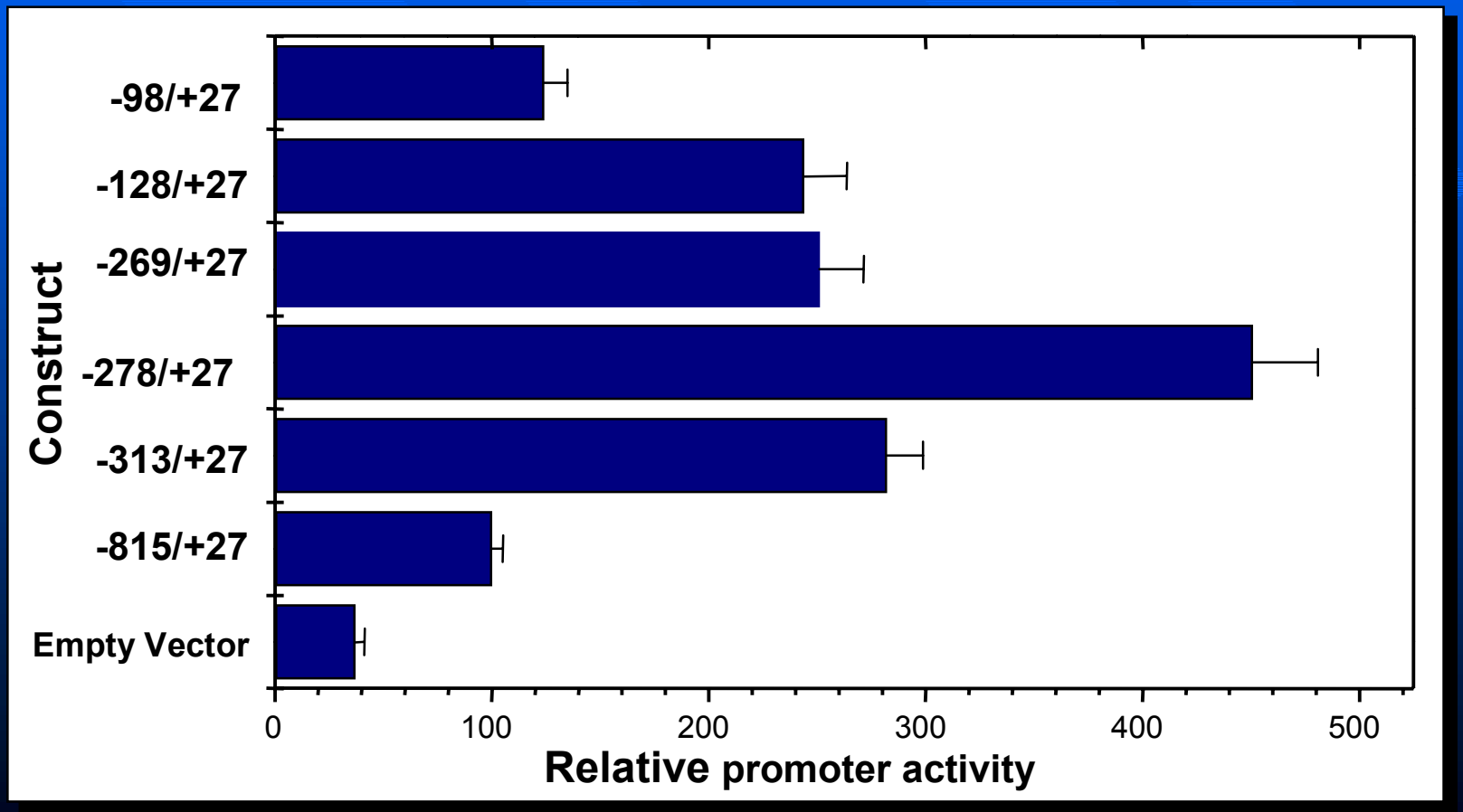
Identification of the regulatory elements in CYP2E1 promoter

- Generation of promoter fragments by PCR



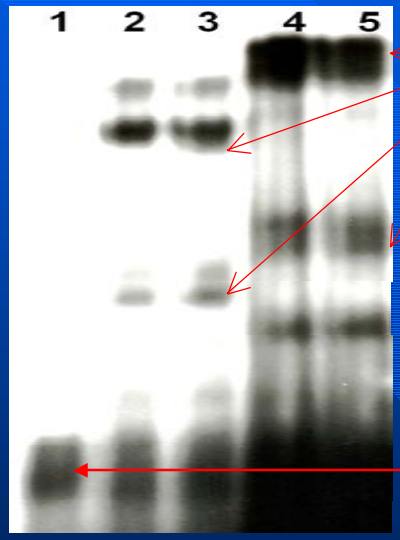
- Ligation of the fragments into pGL-basic vector containing luciferase cDNA (generation of constructs)
- Transfection of hepatoma cells with the constructs
- Measuring promoter activity of the constructs as induction of luciferase activity

Promoter Activity of Plasmid Constructs



Identification of proteins binding to CYP2E1 regulatory elements

1. Electrophoretic Mobility Shift Assay



Shifted bands due to specific liver nuclear extract protein binding

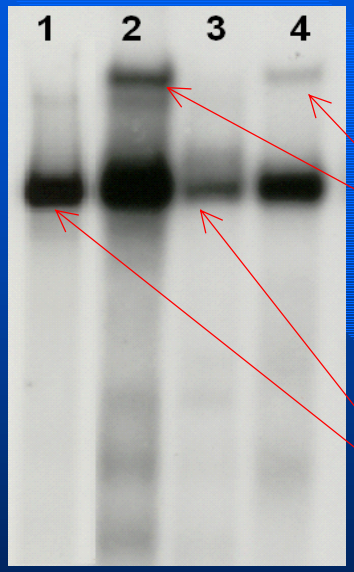
Control probe

Nuclear extract proteins bind to:

- oligonucleotides corresponding to the binding sites for **HNF-1** (lines 2,3)
- A sequence defined as **TGTTCTGACCTCTGGG** (lines 4-5).

Identification of proteins binding to CYP2E1 regulatory elements

2. Gel supershift assay



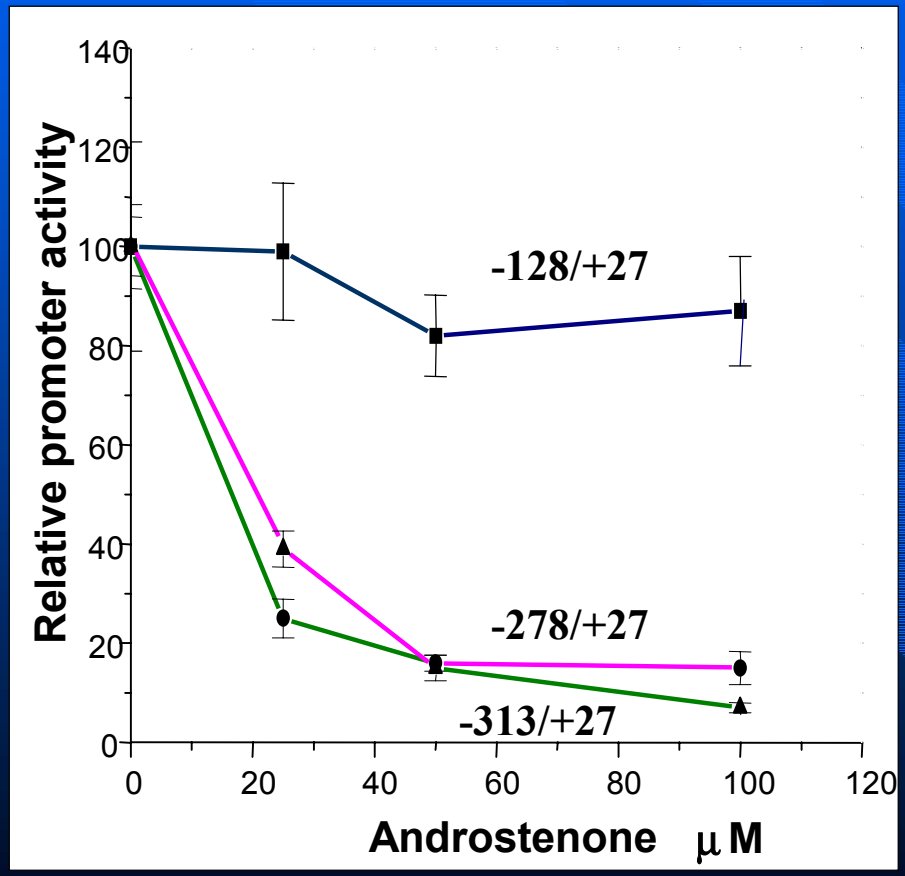
Super-shifted bands

Control EMSA bands. Lanes, 1 & 2, N1; 3 & 4, N4

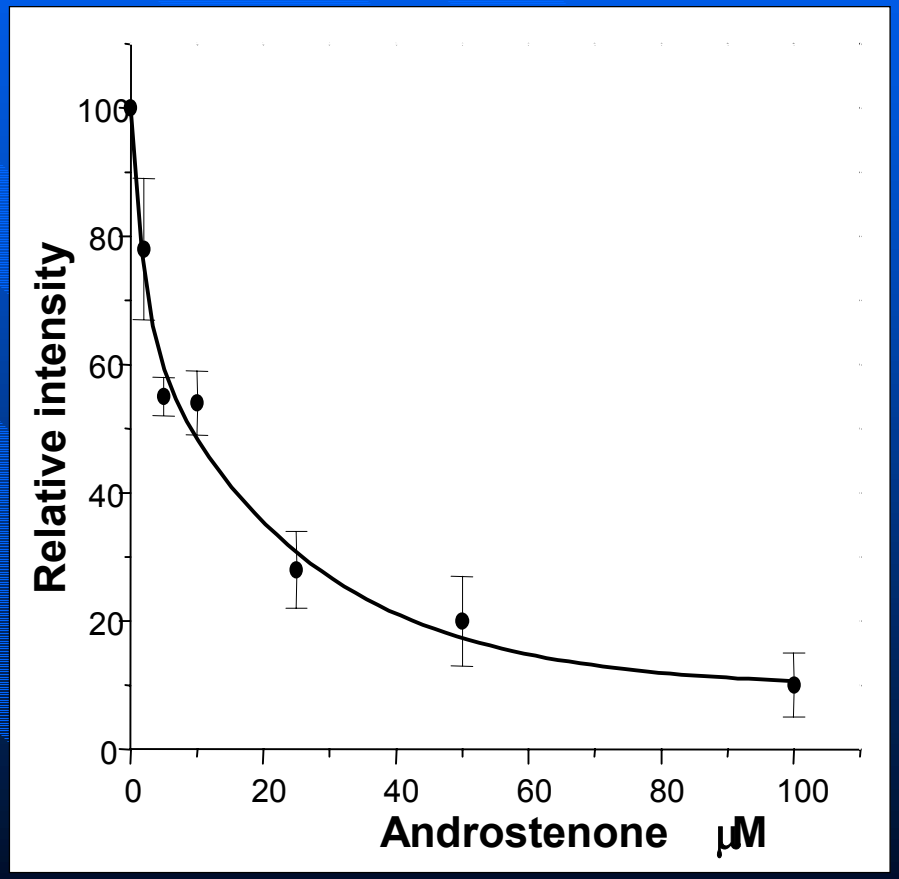
TGTTCTGACCTCTGGG sequence was identified as the transcription factor **COUP-TF1.**

Effect of androstenone on CYP2E1 promoter activity and COUP-TF1 binding

Promoter Activity



COUP-TF1 binding



- **Transcription factors HNF-1 and COUP-TF1 are required for activation of CYP2E1 promoter**

- **Androstenone represses CYP2E1 activity via inhibition of binding COUP-TF1 (but not HNF-1)**

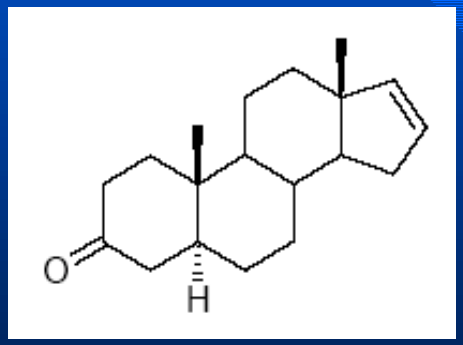
Objectives

1. To investigate the molecular mechanism regulating CYP2E1 expression

2. To investigate the relationship between hepatic androstenone metabolism and androstenone accumulation in adipose tissue.

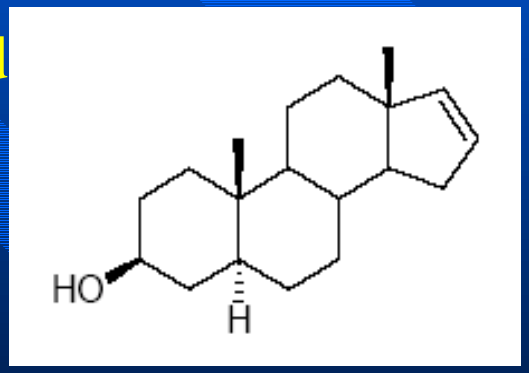
Androstenone metabolism in pigs liver

- The reaction requires NADH
- The major product of the reaction is 3-beta-androstenol



Androstenone

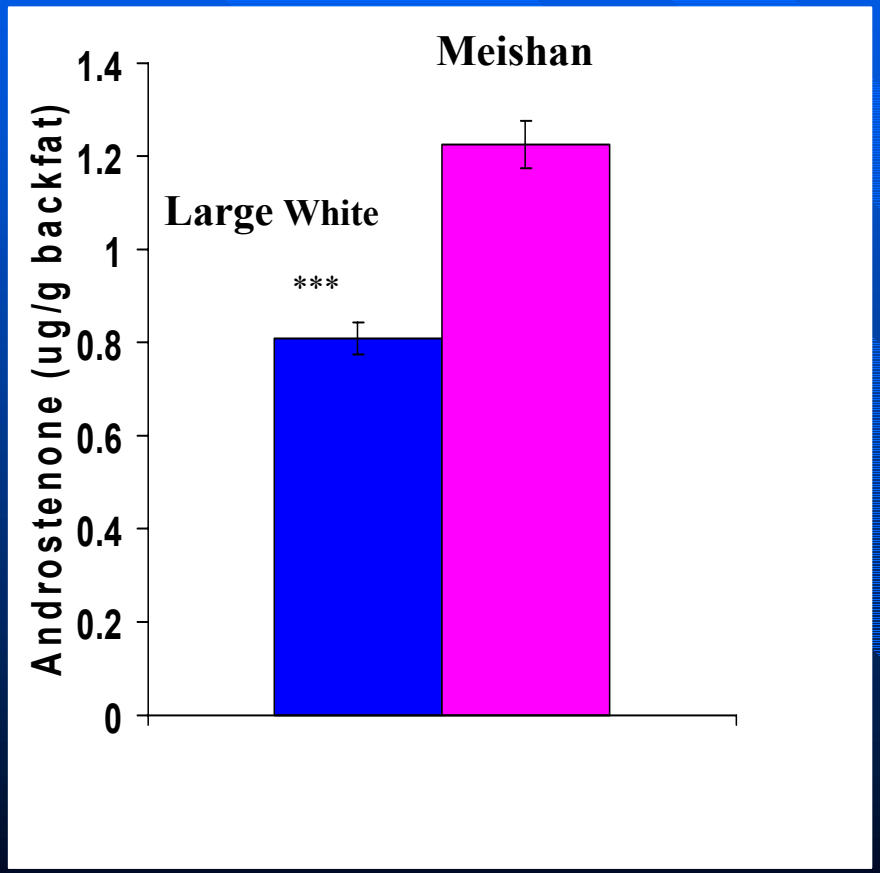
3-beta-hydroxysteroid dehydrogenase (HSD)



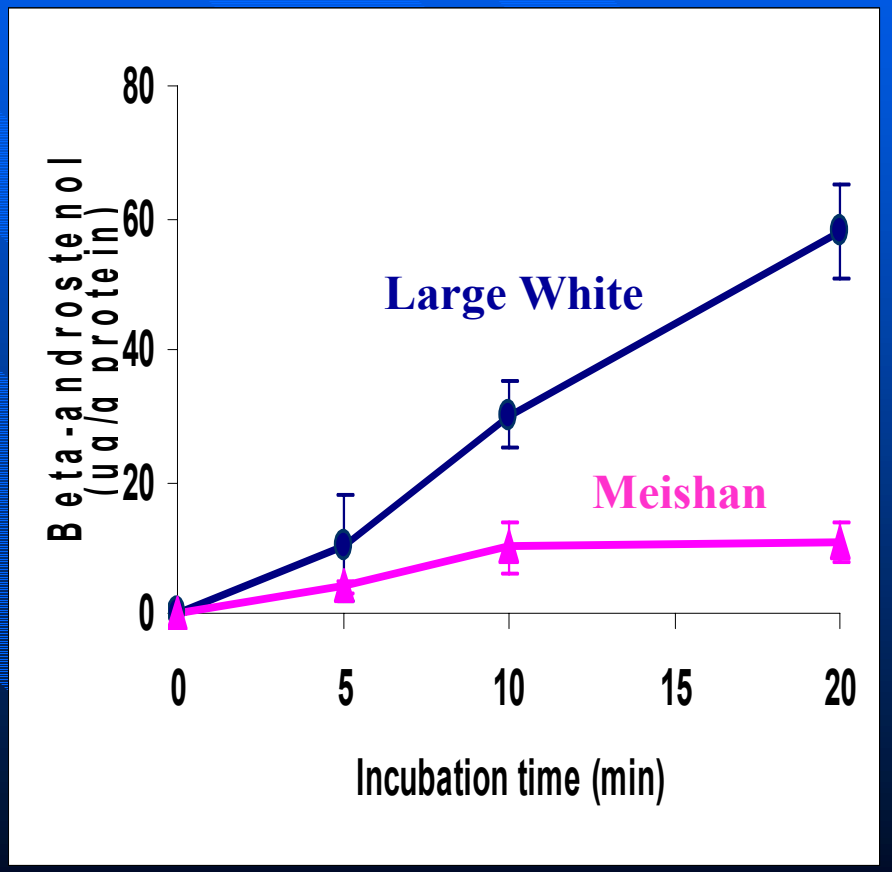
3-beta-androstenol

- The reaction can be inhibited by a specific HSD inhibitor trilostane

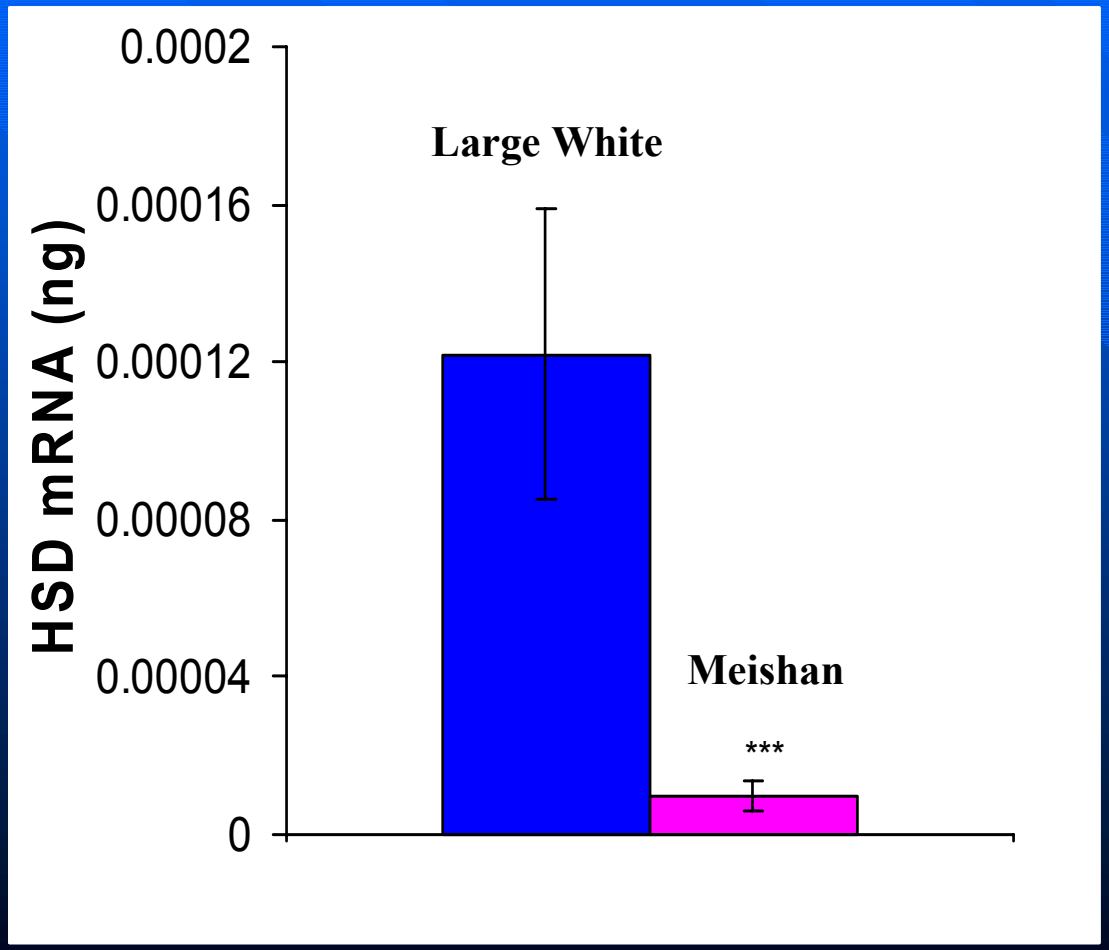
Androstenone level in backfat



Rate of androstenone metabolism in liver

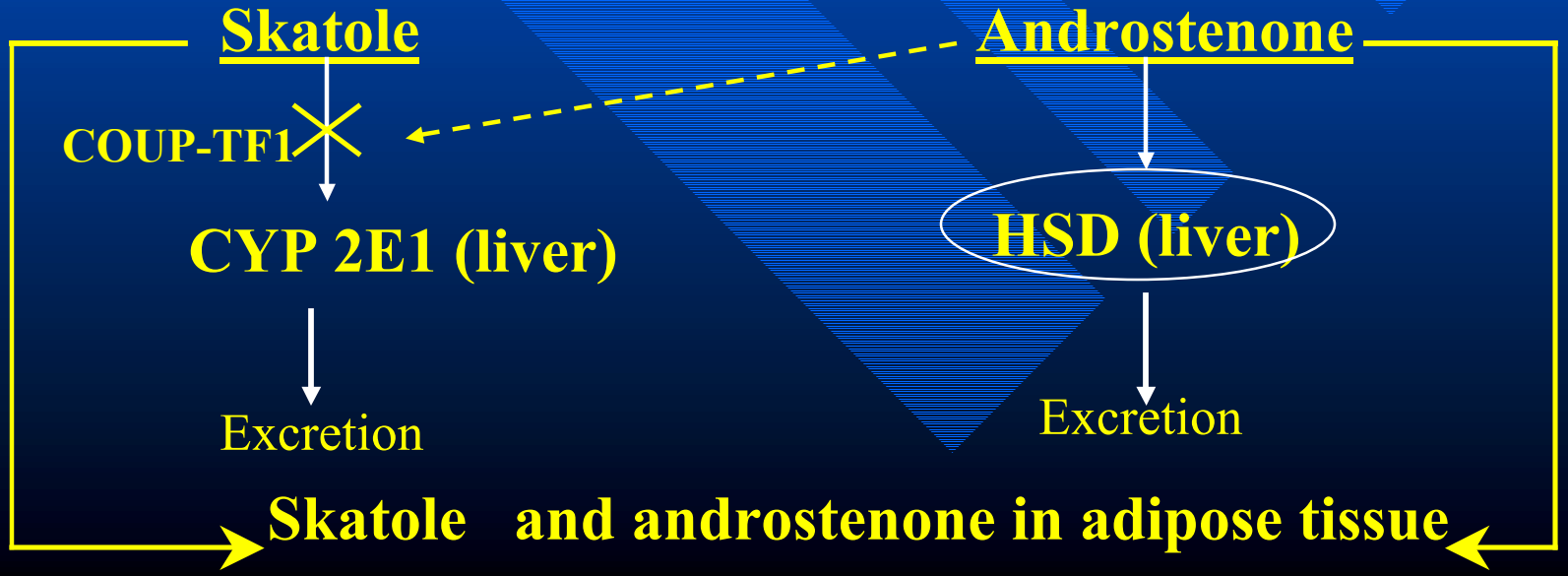


HSD expression in pig liver



Summary

- HNF-1 and COUP-TF1 activate CYP2E1 promoter
- Androstenone is metabolised via HSD in pig liver
- Low expression of HSD in liver is related to a high androstenone accumulation in adipose tissue
- Androstenone represses CYP2E1 promoter activity via inhibition of binding of COUP-TF1



Acknowledgments

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