Expression of 11² -HSD1 mRNA in adipose tissue of dairy cows during the periparturient period

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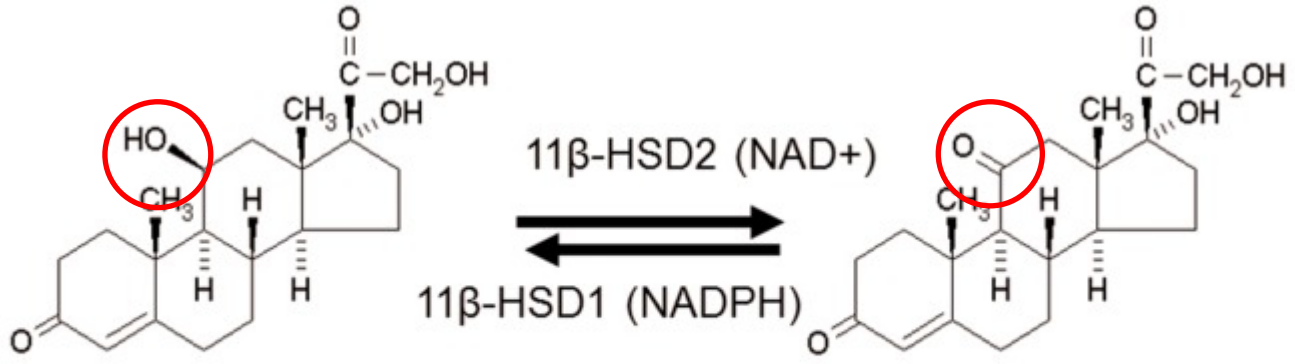
Backgrounds

11-beta-hydroxysteroid-dehydrogenase1 (**11ßHSD1**):

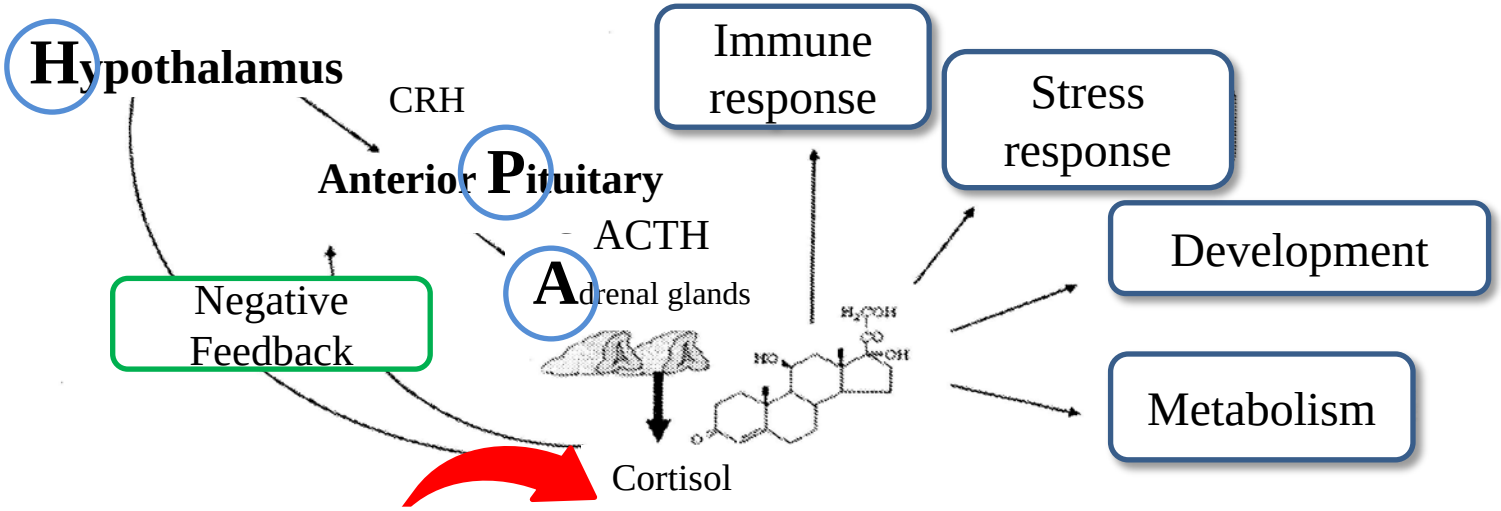
- enzyme (reductase)
- catalyzes the NADPH-dependent reduction of cortisone to cortisol



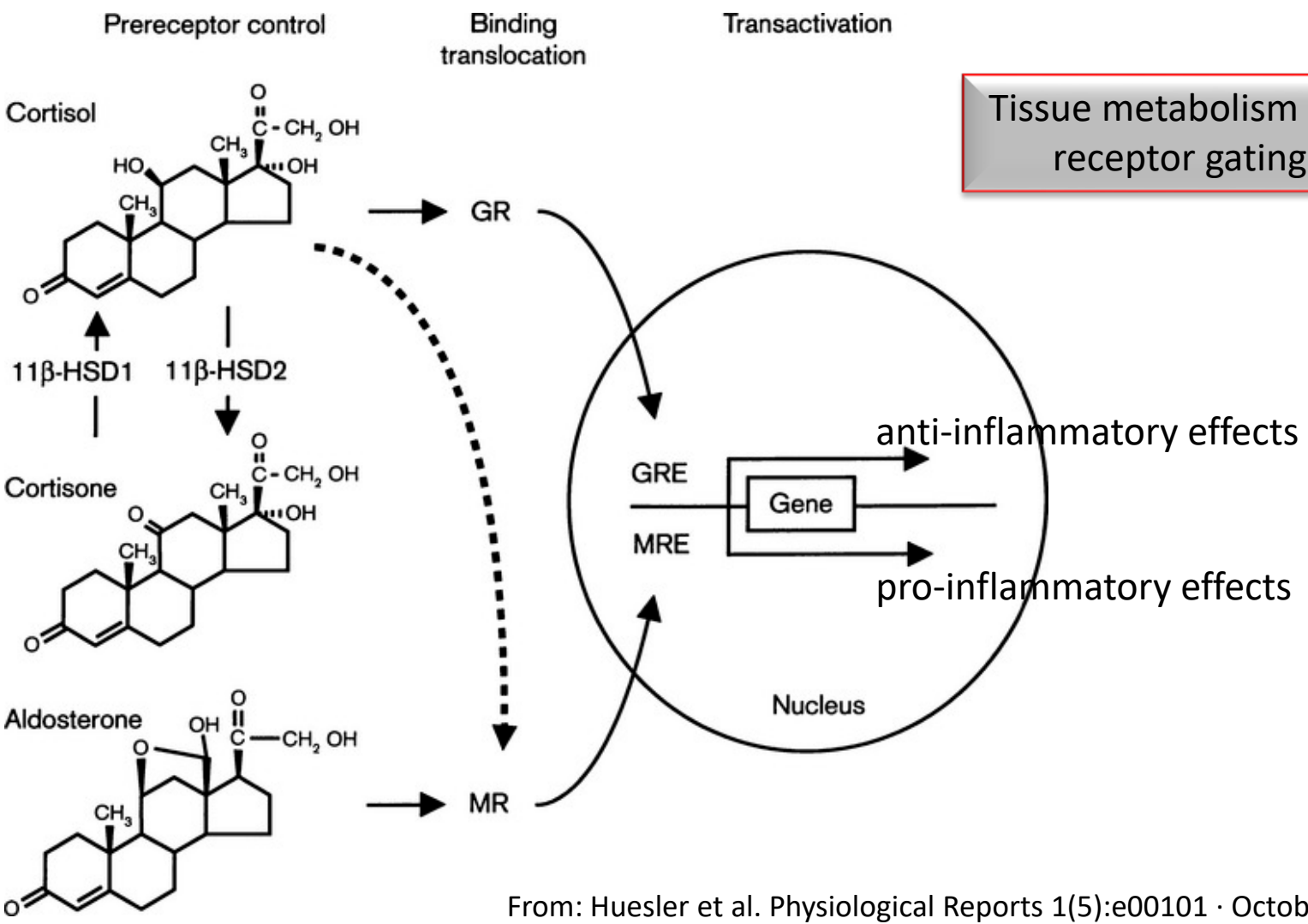
Cortisol
(Biologically active)

Cortisone
(Biologically inactive)

Backgrounds:
HPA axis and tissue metabolism



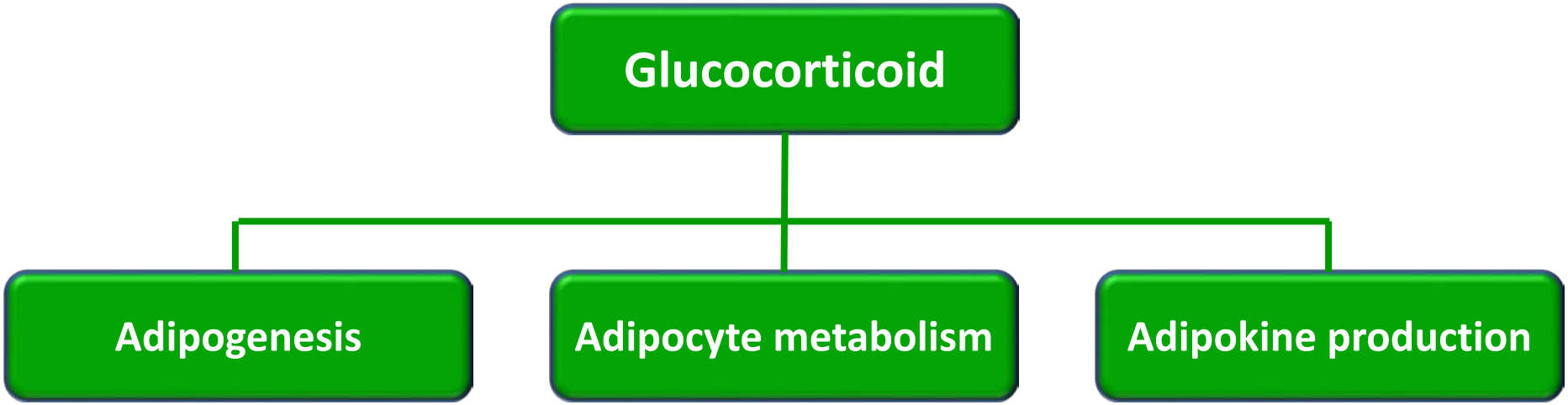
From Andrew & Homer, 2016. Biochem. Soc. Trans 44, 645-651



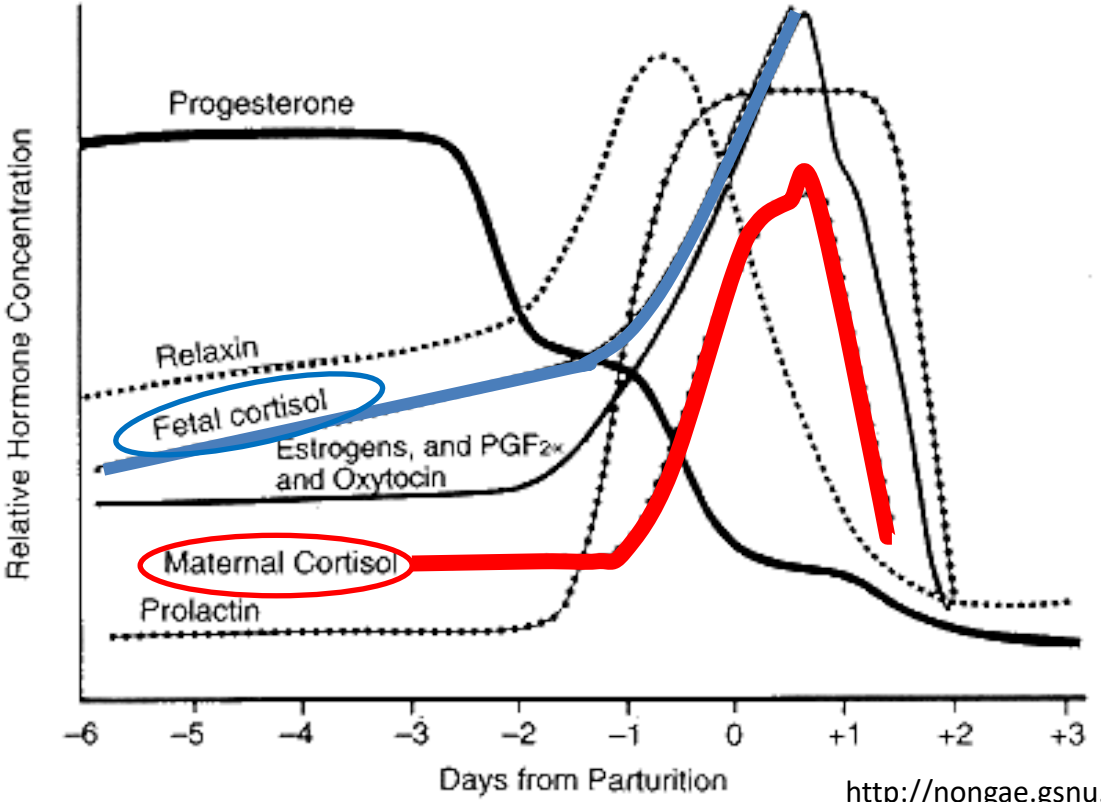
Tissue metabolism and receptor gating

From: Huesler et al. Physiological Reports 1(5):e00101 · October 2013

Glucocorticoid effects in adipose tissue



Glucocorticoids in the circulation around calving



http://nongae.gsnu.ac.kr/~cspark/teaching/chap9.html

Hypothesis and Objectives

Hypothesis: Calving affects 11² -HSD1 mRNA in adipose tissue

Objectives:

- To assess the mRNA expression of 11² -HSD1
- To test the effects of different intensities of lipo-mobilization
- To characterize the time course of mRNA abundance during late pregnancy and early lactation
- To compare this time course in subcutaneous versus visceral fat

Experimental design

20 Holstein cows,
same diet

Day 42 ante partum

Biopsy samples
Sc fat
Rp fat

n = 10
High concentrate diet (**HC**)
60 : 40 concentrate : roughage

qPCR → Stats

n = 5 with niacin*	n = 5 without
HC⁺	**HC⁻**

n = 10
low concentrate diet (**LC**)
30 : 70 concentrate : roughage

n = 5 with niacin*	n = 5 without
LC⁺	**HC⁻**

Day 1 post partum

Day 21 p.p.

Day 100 p.p.

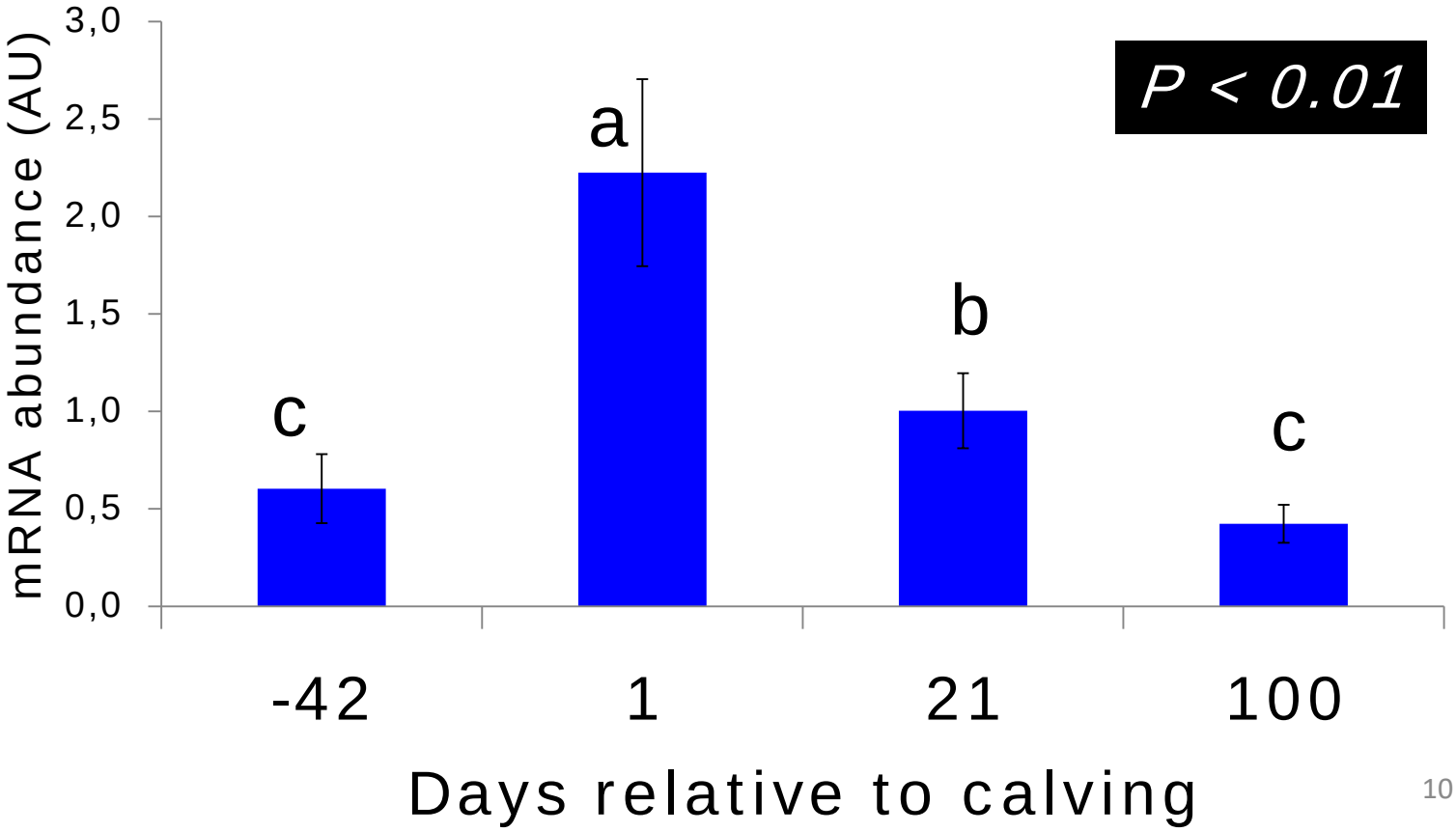
All groups on same diet
50 : 50

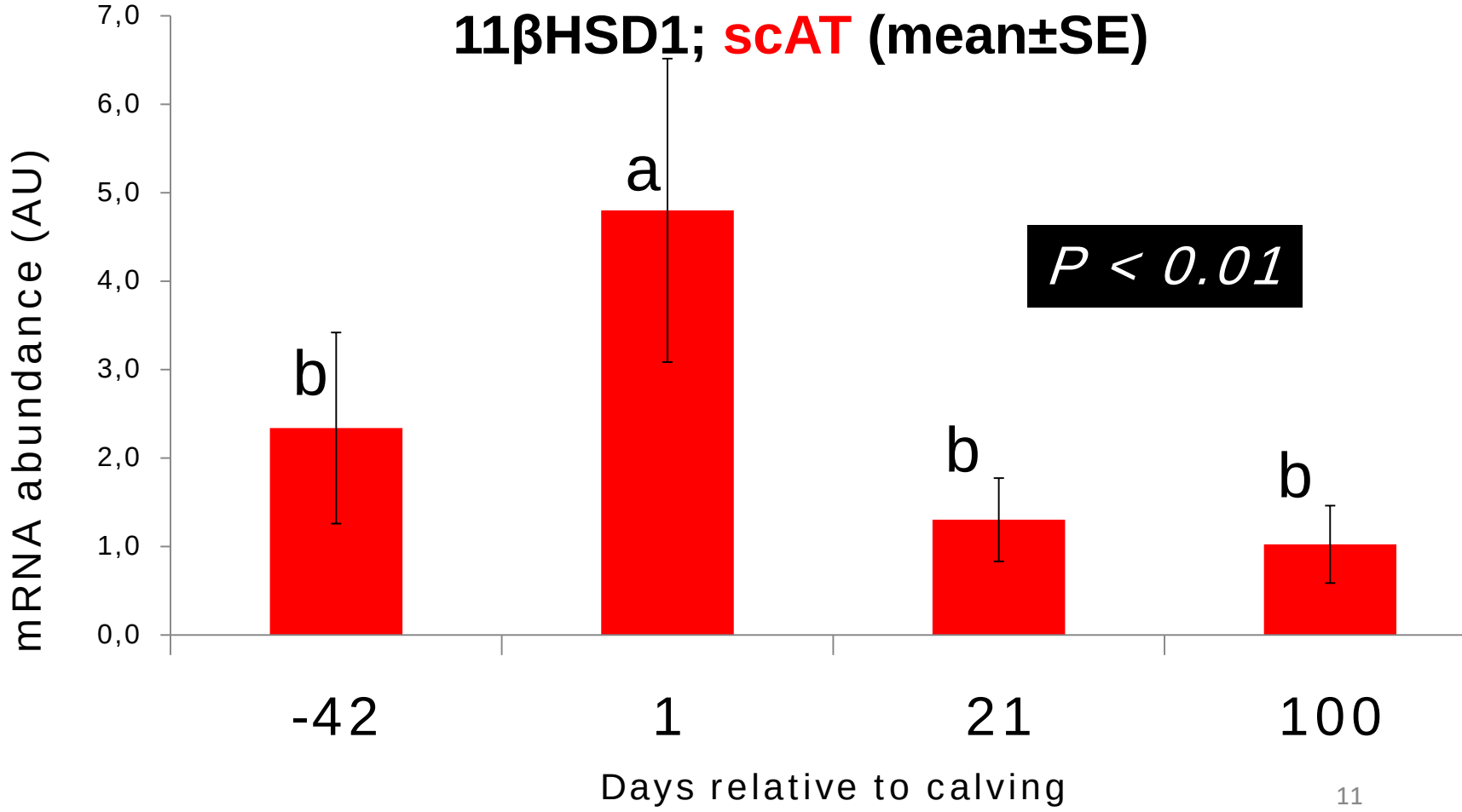
*24 g/d/cow

Results

- 11² HSD1 mRNA abundance in **rpAT** and **scAT** was not affected by diet
- Longitudinal changes:

11² HSD1; rpAT (mean±SE)




11βHSD1; scAT (mean±SE)
mRNA abundance (AU)
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
a
b
b
b
P < 0.01
-42
1
21
100
Days relative to calving

Context setting

11ßHSD1 mRNA is expressed in bovine adipose tissue (bAT)

Earlier findings in bovine adipose tissue (primiparous cows, comparison of DIM 1, 42 & 105; Friedauer et al., 2015):

- 11ßHSD1 protein (IHC) localized in mature adipocytes
- 11ßHSD1 activity in tissue homogenates: no change with DIM
- Glucocorticoid receptor (IHC) is localized in mature adipocytes
- Mineralocorticoid receptor (IHC) is localized in the stroma vascular fraction and number of positive cells correlated with Pref-1 expression (= preadipocyte marker)

→ Metabolic and anti-inflammatory rather than differentiating effects of cortisol in mature adipocytes

→ potentially paracrine adipogenetic effects of cortisol secreted by mature adipocytes

Conclusions

The patterns observed for 11ßHSD1 mRNA abundance are in support of increased tissue concentrations of cortisol in adipose tissue around calving

Sustained elevation of 11βHSD1 mRNA abundance in retroperitoneal vs. subcutaneous adipose tissue on d 21 p.p. may indicate greater generation of cortisol in visceral than in subcutaneous adipose tissue

Metabolic and anti-inflammatory effects are assumed as main biological consequences

Thank you

qPCR and analysis of data

- **Primers**: as published by Tetsuka et al. (2010)

Positive Control: liver from slaughterhouse

❑ **Reference genes for rpAT**

- ✓ LRP10
- ✓ EMD
- ✓ POLR2A

❑ **Reference genes for scAT**

- ✓ EIF3K
- ✓ LRP10
- ✓ MARVELD
- ✓ EMD MAR
- ✓ Pol2

Papers in Press. Published February 26, 2009 as doi:10.1373/clinchem.2008.112797
The latest version is at http://www.clinchem.org/cgi/doi/10.1373/clinchem.2008.112797

Clinical Chemistry 55:4
000–000 (2009)

Reviews

The MIQE Guidelines:
Minimum Information for Publication of Quantitative Real-Time PCR Experiments

Stephen A. Bustin,[1*] Vladimir Benes,[2] Jeremy A. Garson,[3,4] Jan Hellemans,[5] Jim Huggett,[6] Mikael Kubista,[7,8] Reinhold Mueller,[9] Tania Nolan,[10] Michael W. Pfaffl,[11] Gregory L. Shipley,[12] Jo Vandesompele,[5] and Carl T. Wittwer[13,14]

BACKGROUND: Currently, a lack of consensus exists on how best to perform and interpret quantitative real-time PCR (qPCR) experiments. The problem is exacerbated by a lack of sufficient experimental detail in many publications, which impedes a reader's ability to evaluate critically the quality of the results presented or to repeat the experiments.

CONTENT: The Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines target the reliability of results to help ensure the integrity of the scientific literature, promote consistency between laboratories, and increase experimental transparency. MIQE is a set of guidelines that describe the minimum information necessary for evaluating qPCR experiments. Included is a checklist to accompany the initial submission of a manuscript to the publisher. By providing all relevant experimental conditions and assay characteristics, reviewers can as-

SUMMARY: Following these guidelines will encourage better experimental practice, allowing more reliable and unequivocal interpretation of qPCR results.

The fluorescence-based quantitative real-time PCR (qPCR)[15] *(1–3)*, with its capacity to detect and measure minute amounts of nucleic acids in a wide range of samples from numerous sources, is the enabling technology par excellence of molecular diagnostics, life sciences, agriculture, and medicine *(4, 5)*. Its conceptual and practical simplicity, together with its combination of speed, sensitivity, and specificity in a homogeneous assay, have made it the touchstone for nucleic acid quantification. In addition to its use as a research tool, many diagnostic applications have been developed, including microbial quantification, gene dosage determination, identification of transgenes in genetically modified foods, risk assessment of cancer recurrence, and

- **qbase out put:** CNRQ = *Calibrated Normalized Relative Quantities*
- **Statistical Analyses** (SAS): Mixed Procedure, Repeated Measure