LEIBNIZ INSTITUTE
FOR FARM ANIMAL BIOLOGY

Nutrient signalling receptors for free fatty acids and hydroxycarboxylic acids in farm animals

Manfred Mielenz

Nutritional Physiology
Oskar Kellner

66th EAAP ANNUAL MEETING – Warsaw, Poland 2015

LEIBNIZ INSTITUTE
FOR FARM ANIMAL BIOLOGY

Warsaw 31st of August to 4th of September

Definition of “nutrient sensing”

- **Ability to recognize and respond to macronutrients linked to:**
  energy metabolism and biomass production

- Carbohydrates
- Fatty acids
- Amino acids
- Beta-hydroxybutyric acid (BHBA) and lactate

Definition of “nutrient sensing”

- Ability to recognize and respond to macronutrients linked to:
  energy metabolism and biomass production

- Direct impact on metabolism by their function as substrates
- Effects based on receptor binding
  - Nuclear receptor binding
  - Membrane receptor binding

Scope and significance of the topic

- **Nutrient scarcity: selection of effective mechanisms in nutrient sensing**

  Efeyan et al., 2015, Nature

- **Difficulties to find an area of cell biology in which lipids do not have important or key roles as signalling and regulatory molecules**

  Hannun & Obeid, 2008, Nature Reviews Mol Cell Biol

- **Relatively less knowledge on direct nutrient sensing-mechanisms**

  Efeyan et al., 2015, Nature

Scope and significance of the topic

1

- **Fatty acids besides their role in energy metabolism act as important signalling molecules e.g. during fasting**

de Lange et al., 2007, FASEB J, modified

Direct Regulatory Effect of Ketones on Lipolysis and on Glucose Concentrations in Man

Senior & Loridan

controlling lipo

Volume 21, number 2 FEBS LETTERS March 1972

EFFECTS OF VOLATILE FATTY ACIDS, KETONE BODIES, GLUCOSE, AND INSULIN ON LIPOLYSIS IN BOVINE ADIPOSE TISSUE

Stef H.M. METZ and Simon G. VAN DEN BERGH

Laboratory of Veterinary Biochemistry, State University of Utrecht, Utrecht, The Netherlands

Family of free fatty acid binding receptors

Ligands:

- Medium-chain fatty acids
- Long-chain fatty acids (**LCFA**)
- Short-chain fatty acids (**SCFA**)

Family of free fatty acid binding receptors

2

Medium- and **LCFA**

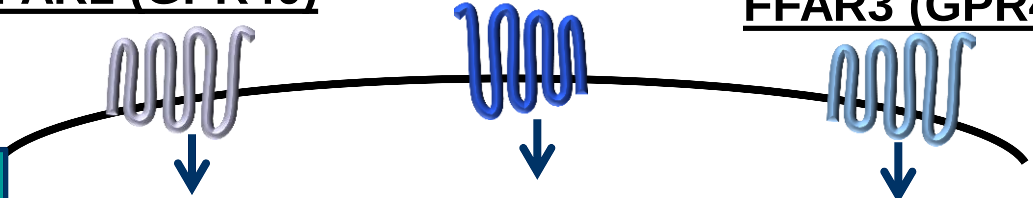
SCFA

FFAR4 (GPR120)

FFAR1 (GPR40)

FFAR2 (GPR43)

FFAR3 (GPR41)



Adipogenesis, lipid accumulation (Ichimura et al., 2014)

$G\alpha_{q/11}$

$G\alpha_{q/11}$

$G\alpha_{i/o}$

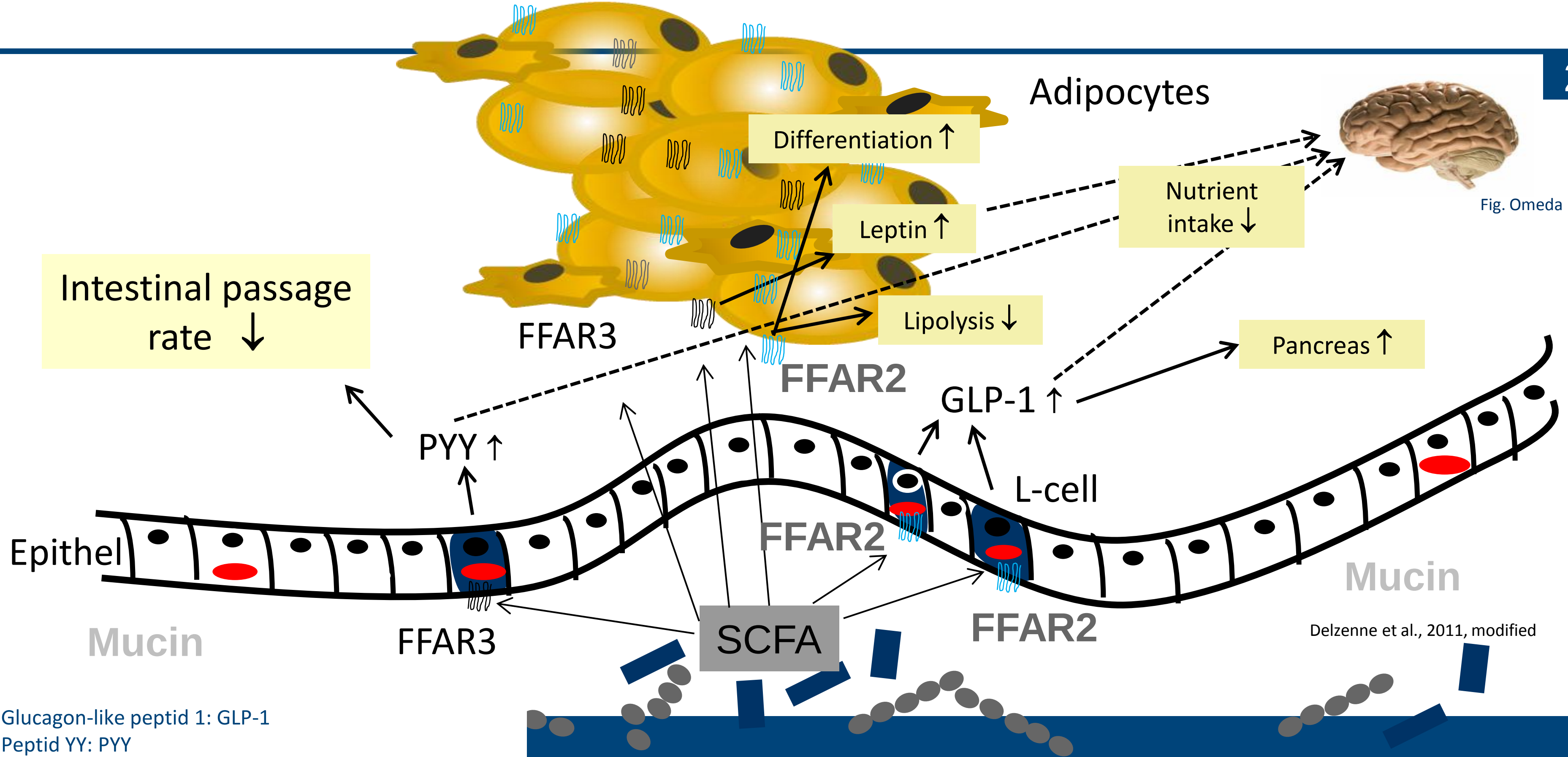
Inhibitory G-protein coupled

Link to the innate immune system (Brown et al., 2003)

e.g. FFAR4 but not FFAR1 on human eosinophil granulocytes (Konno et al., 2015)

Stoddart et al., 2008, modified

Intestinal effects of FFAR2 and FFAR3 activation



Glucagon-like peptid 1: GLP-1
Peptid YY: PYY
Peroxisome proliferator-activated receptor γ: PPARγ

Intestinal expression of FFAR1 and FFAR4

FFAR1 →

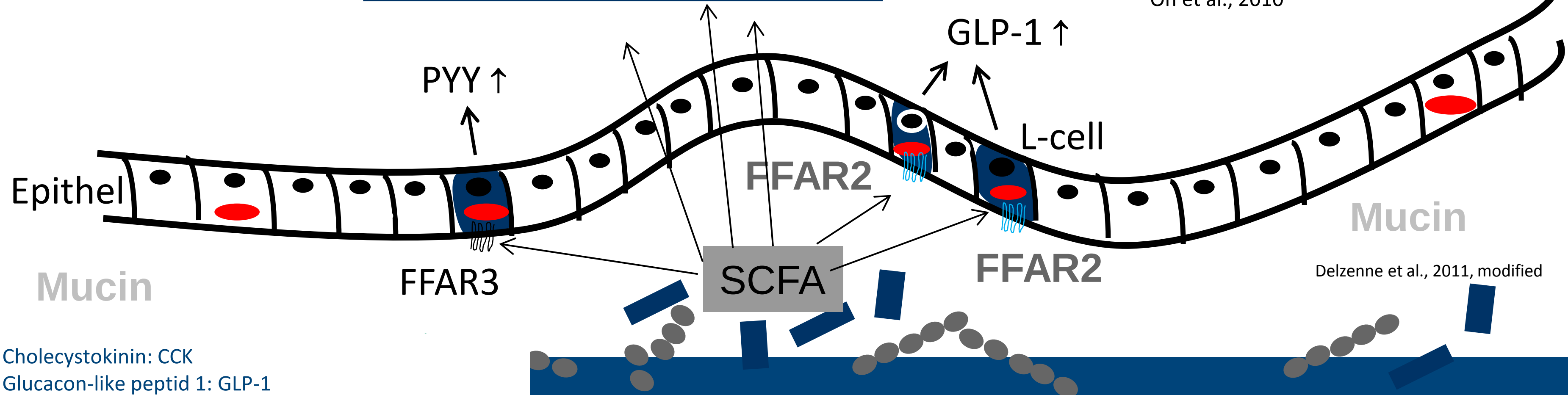
K cells: GIP
L cells: GLP-1
I cells: CCK

Hara et al., 2014

FFAR4 →

L cells: GLP-1
I cells: CCK

Hirasawa et al., 2005; Tanaka et al., 2008
Oh et al., 2010

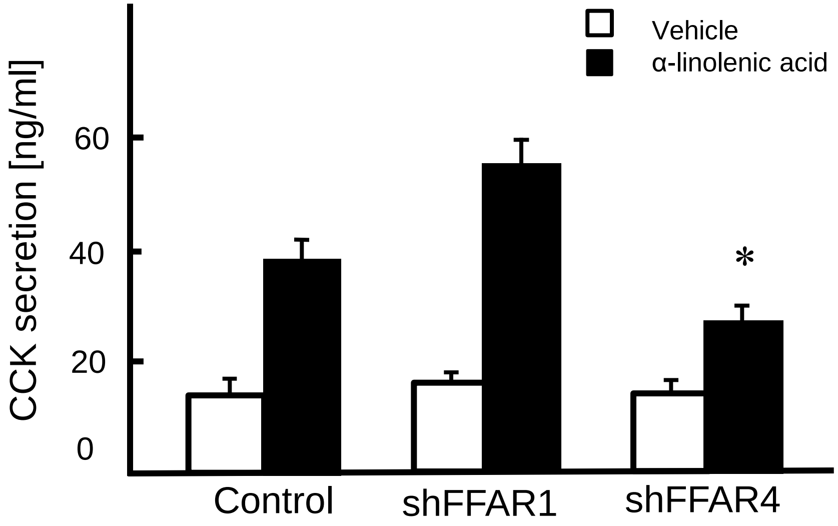


Delzenne et al., 2011, modified

Cholecystokinin: CCK
Glucacon-like peptid 1: GLP-1
Glucose-dependent insulinotropic peptide : GIP

Intestinal expression of FFAR1 and FFAR4

FFAR4 but not FFAR1 affects CCK secretion



Tanaka et al., 2008, modified

Cholecystokinin: CCK

Family of hydroxycarboxylic acid binding receptors

Ligands:

- BHBA
- Polyphenolic acids
- Nicotinic acid
- Lactate
- Butyrate

Studies on the Effect of Nicotinic Acid on Catecholamine Stimulated Lipolysis in Adipose Tissue in Vitro

By

LARS A. CARLSON

1963

Family of hydroxycarboxylic acid binding receptors

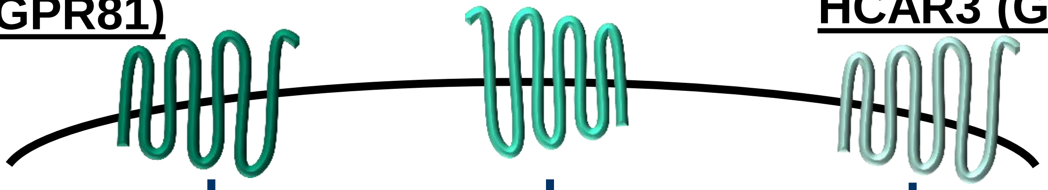
Lactate

Hydroxycarboxylic acids

HCAR1 (GPR81)

HCAR2 (GPR109A)

HCAR3 (GPR109B) (only primates!)



Inhibitory
G-protein coupled
receptors

$G\alpha_{i/o}$

$G\alpha_{i/o}$

$G\alpha_{i/o}$

Antilipolysis

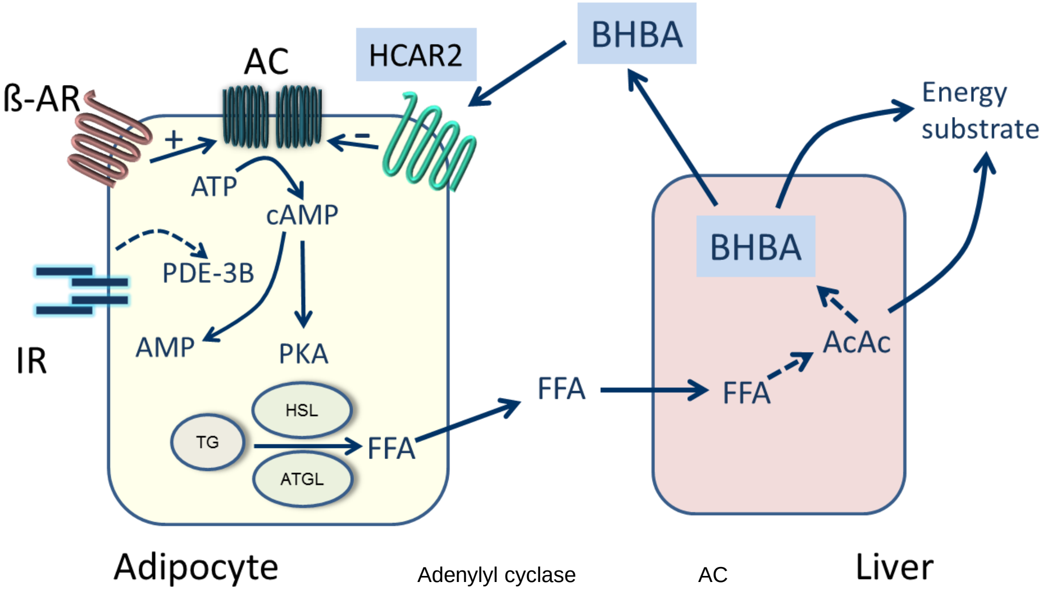
Antilipolysis,
innate immune system

Antilipolysis, innate
immune system

Offermanns et al., 2011, modified

Feedbackmechanism of lipolysis and the role of HCAR2

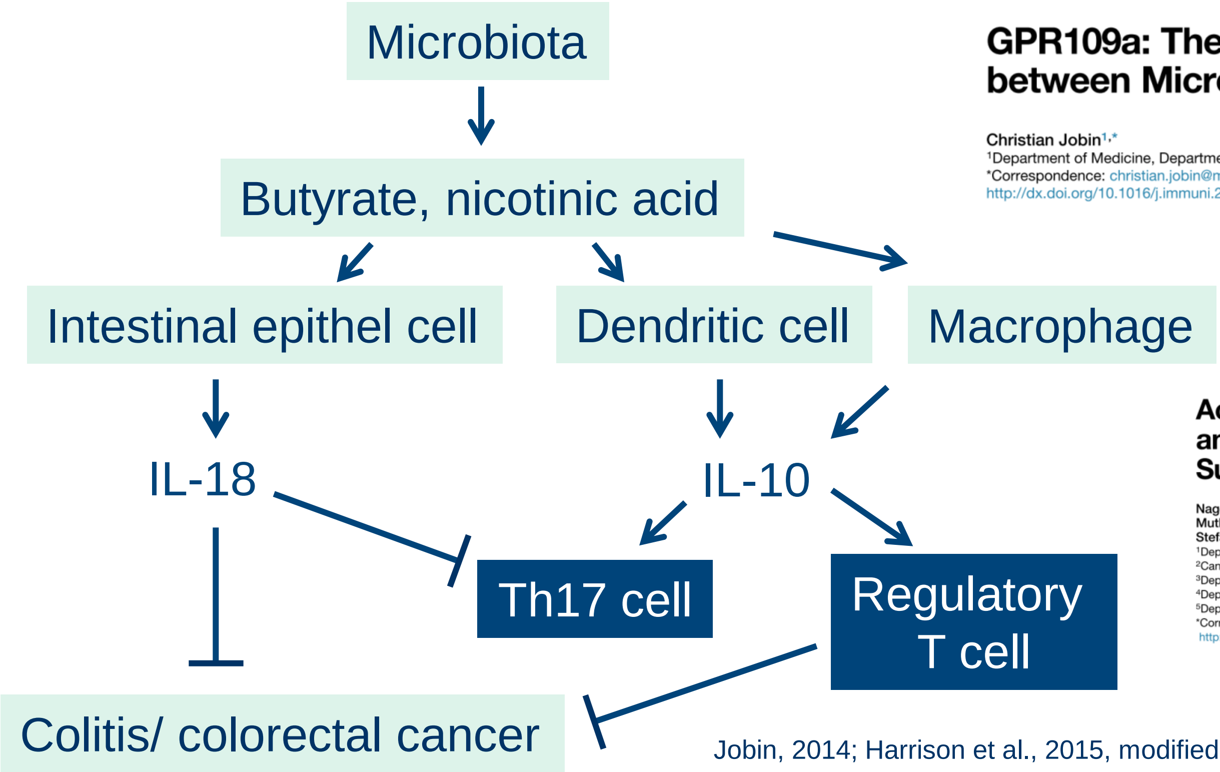
- Inhibitory G-Protein
- Antilipolytic activity
- Antagonistic activity to adrenergic stimulation of lipolysis
- Fine tuning of lipolysis



Adenylyl cyclase	AC
Adipocyte triglyceride lipase	ATGL
ß-adrenergic receptor	ß-AR
Hormone sensitive Lipase	HSL
Insulin receptor	IR
Protein kinase A	PKA
Phosphodiesterase 3B	PD3B

Gille et al., 2008, modified

Importance of the intestinal expression of hydroxycarboxylic acid binding receptor HCAR2 (GPR109A)



Jobin, 2014; Harrison et al., 2015, modified

GPR109a: The Missing Link between Microbiome and Good Health?

Christian Jobin[1,*]
[1]Department of Medicine, Department of Infectious Diseases & Pathology, University of Florida, Gainesville, FL 32611, USA
*Correspondence: christian.jobin@medicine.ufl.edu
http://dx.doi.org/10.1016/j.immuni.2013.12.009

Jobin, 2014

Activation of Gpr109a, Receptor for Niacin and the Commensal Metabolite Butyrate, Suppresses Colonic Inflammation and Carcinogenesis

Nagendra Singh,[1,2,*] Ashish Gurav,[1] Sathish Sivaprakasam,[1] Evan Brady,[1] Ravi Padia,[1] Huidong Shi,[1,2] Muthusamy Thangaraju,[1,2] Puttur D. Prasad,[1,2] Santhakumar Manicassamy,[2] David H. Munn,[2,3] Jeffrey R. Lee,[4] Stefan Offermanns,[5] and Vadivel Ganapathy[1,2,*]
[1]Department of Biochemistry and Molecular Biology, Medical College of Georgia, Georgia Regents University, Augusta, GA 30912, USA
[2]Cancer Research Center, Georgia Regents University, Augusta, GA 30912, USA
[3]Department of Pediatrics, Medical College of Georgia, Georgia Regents University, Augusta, GA 30912, USA
[4]Department of Pathology, Charlie Norwood Veterans Administration Medical Center, Augusta, GA 30904, USA
[5]Department of Pharmacology, Max-Planck-Institute for Heart and Lung Research, Ludwigstrasse 43, 61231 Bad Nauheim, Germany
*Correspondence: nasingh@gru.edu (N.S.), vganapat@gru.edu (V.G.)
http://dx.doi.org/10.1016/j.immuni.2013.12.007

Singh et al., 2014

Family of Free fatty acid binding receptors in farm animals

Family of free fatty acid binding receptors in pig

- Highest mRNA abundance of FFAR2 and FFAR3 in the distal part of the small intestine

Colocalisation with:

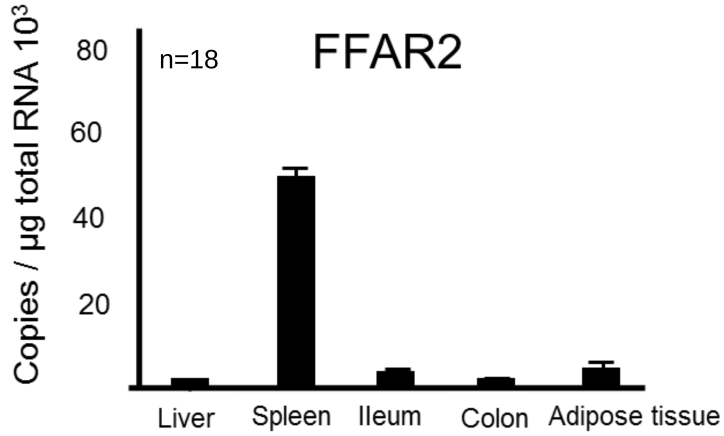
- High PYY mRNA abundance
- Highest AGPTL4 mRNA abundance
- Highest SLC5A8 mRNA abundance

Angiopoietin-like protein 4:	ANGPTL4
Peptide YY:	PYY
Sodium/monocarboxylate-cotransporter 5A8:	SGLT5A8

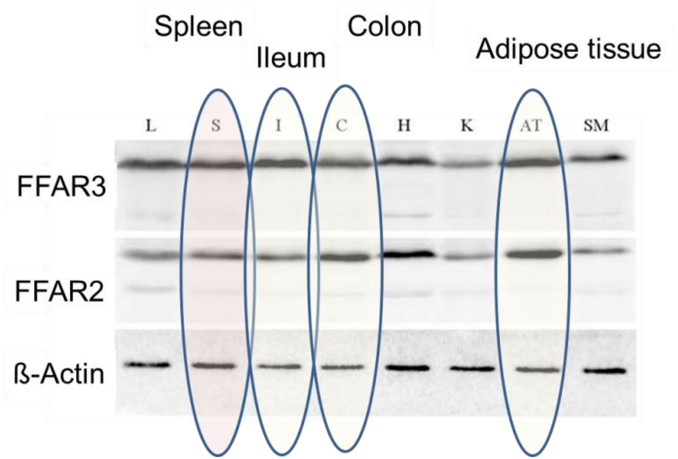
Haenen et al., 2013, modified

Family of free fatty acid binding receptors in tissues of pig

- FFAR2 mRNA mostly linked to the immune system
- FFAR3 mRNA mostly linked to the digestive system



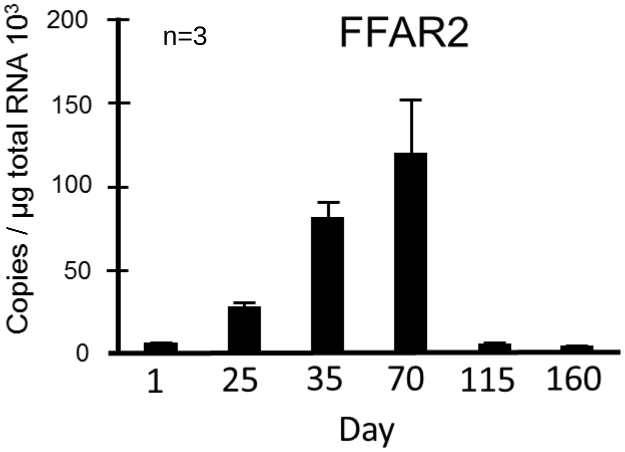
Protein data

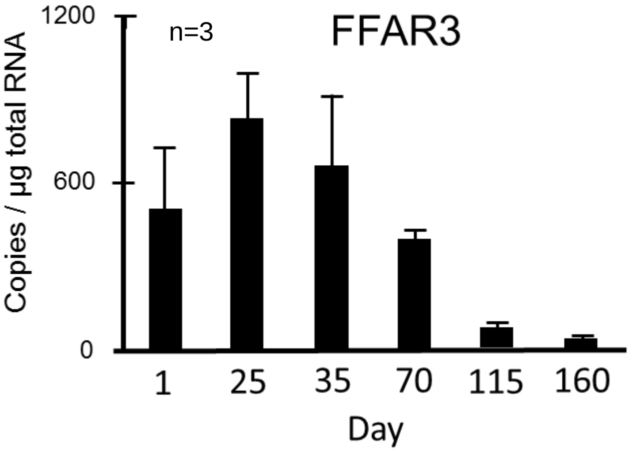


Li et al., 2014, modified

Family of free fatty acid binding receptors in adipose tissue of pig

- Expression in adipose tissue seems to be important up to day 70 postpartum
  → critical role in adipose tissue development?





Li et al., 2014, modified

Family of free fatty acid binding receptors in pig

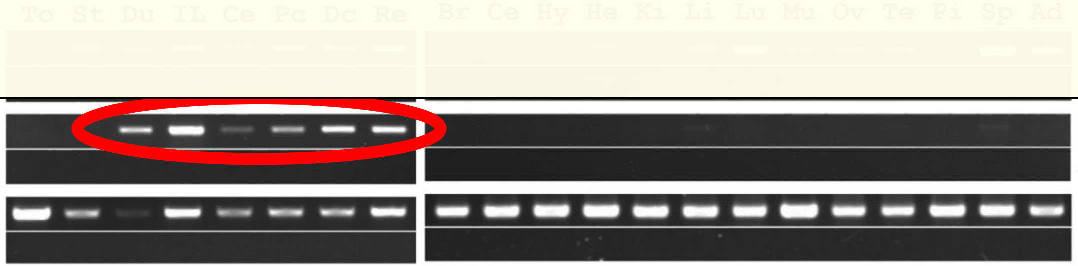
- Two genes for FFAR2 in pigs are existing
- Identification of the **FFAR2-like** gene in 9 of 42 analysed mammalian species
- Differences in their pharmacological characteristics:
  → Activation of FFAR2-like only by **acetate** and **propionate**, not by butyrate

- **Detection of FFAR4 mRNA but not FFAR1 mRNA in**
  - **stomach**
  - **jejunum**
  - **colon**

  of young pigs

Colombo et al., 2012

- Intestinal prevalence of gene expression



Zhang et al., 2014

Detection of free fatty acid binding receptors in chicken

- Expression of FFAR1 in primary hepatocytes *in vitro*

Suh et al., 2008

- 26 paralogs of FFAR2 within the chicken genome

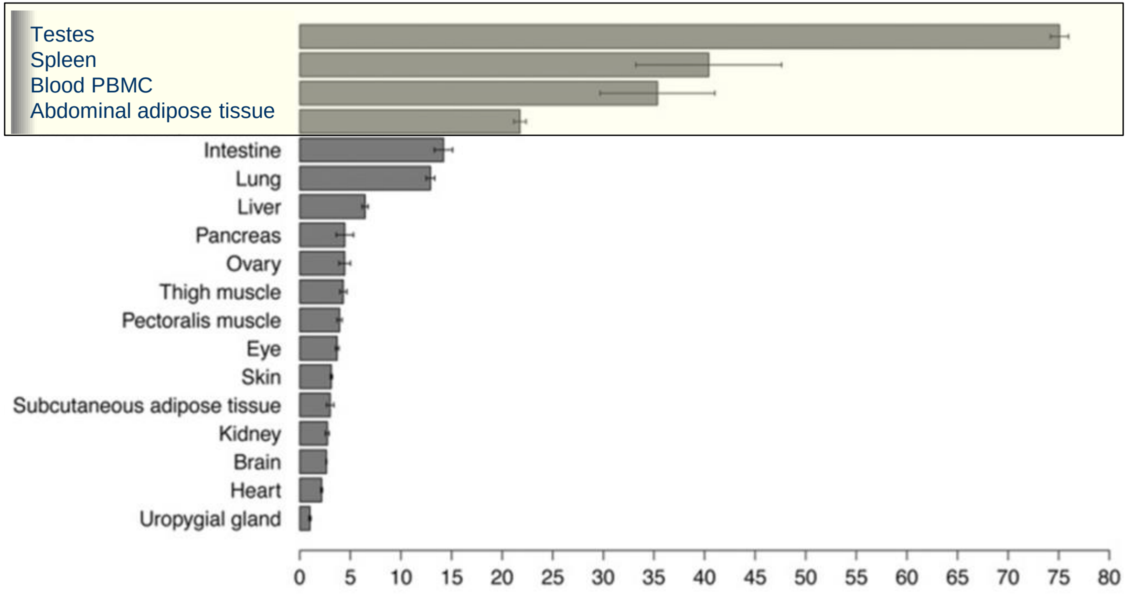
  → chicken specific event, not in finch, turkey, quail!

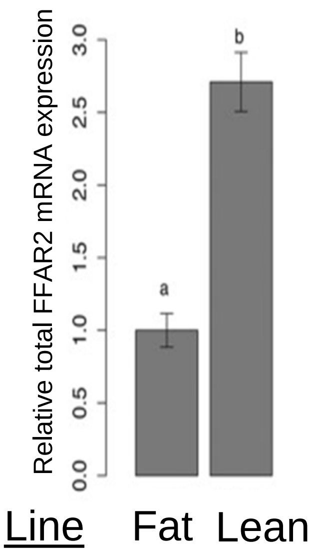
- Recent duplication of FFAR2 paralogs in the chicken genome

Meslin et al., 2015

Detection of free fatty acid binding receptors in chicken

- Low abundance of total FFAR2 mRNA in most tissues (qPCR)

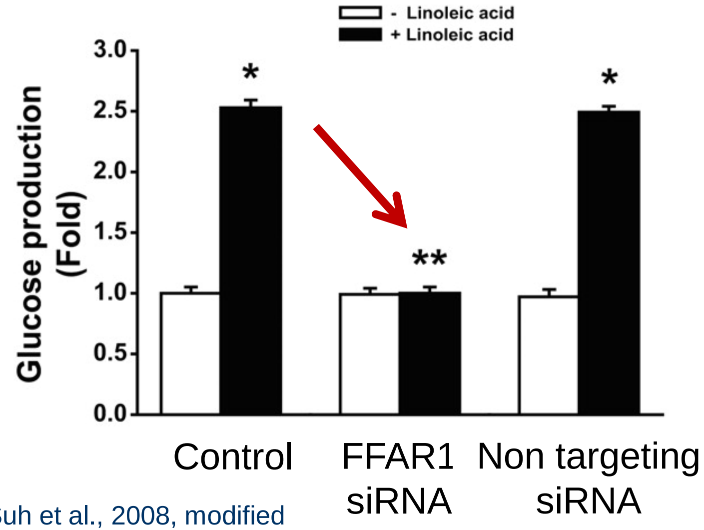




Meslin et al., 2015, modified

Effects of FFAR1 activation in chicken hepatocytes *in vitro*

- Linoleic acid increases hepatic glucose production dose and time dependently



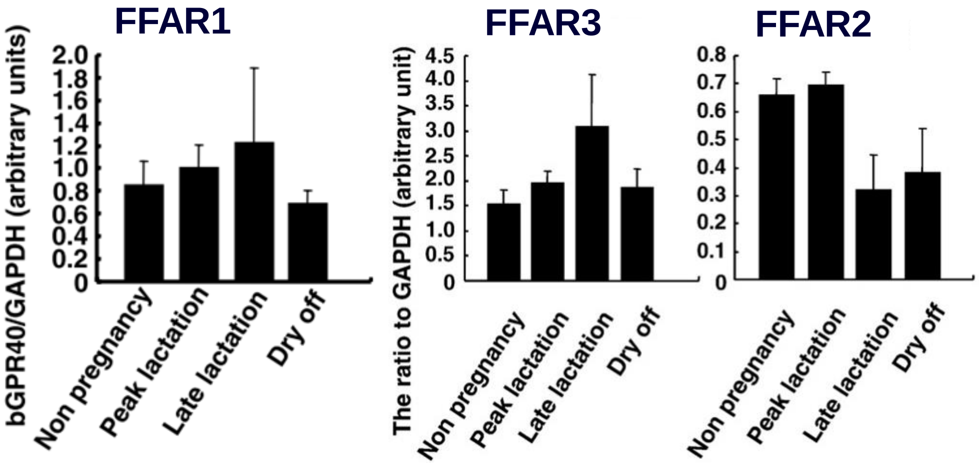
Suh et al., 2008, modified

- No detection of FFAR1 or FFAR3 genes

Meslin et al., 2015

Family of free fatty acid binding receptors in ruminants

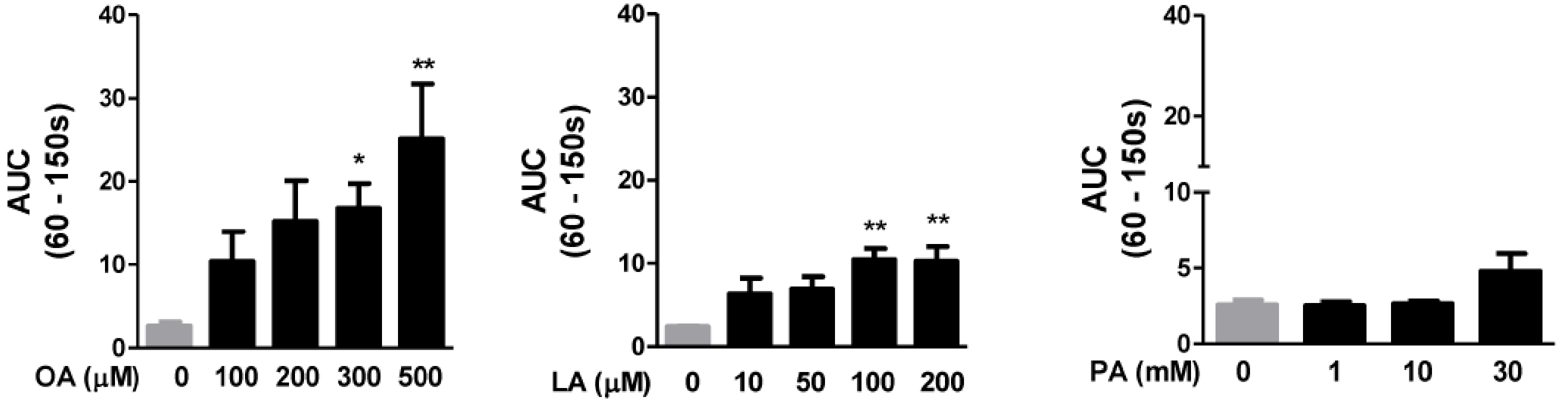
- **FFAR1**, **FFAR2**, **FFAR3** mRNA are expressed in the bovine mammary gland
- Free fatty acids increase intracellular Ca^{2+} mobilisation *in vitro*
- Induction of proliferation of bovine mammary epithel cells *in vitro*



Yonezawa et al., 2008, 2009

Cloning of bovine FFAR1 and activation by LCFA

- Intracellular Ca^{2+} mobilisation increases after stimulation with LCFA in CHO-K1 cells overexpressing the bovine FFAR1



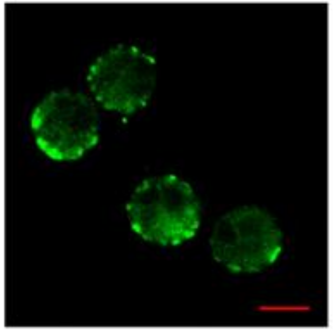
Manosalva et al., 2015

OA Oleic acid; LA linoleic acid; PA propionate

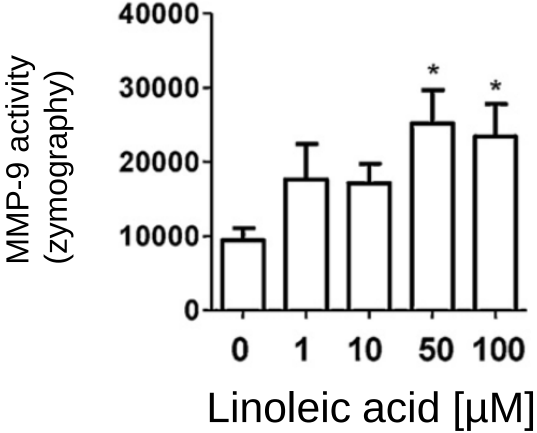
Effects of LCFA on bovine neutrophils

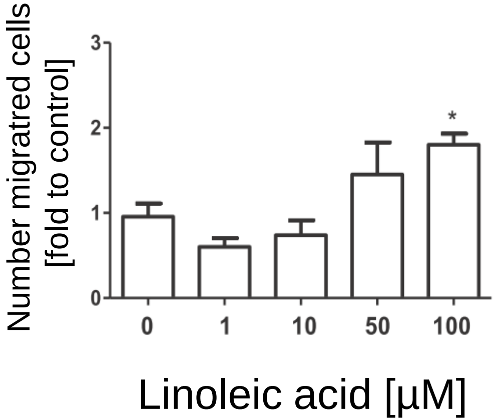
- High NEFA concentrations enhances oxidative burst activity
- Linoleic acid induces MMP-9 release and chemotaxis
- FFAR1 activation induces reactive oxygen species production

FFAR1



Hidalgo et al., 2011



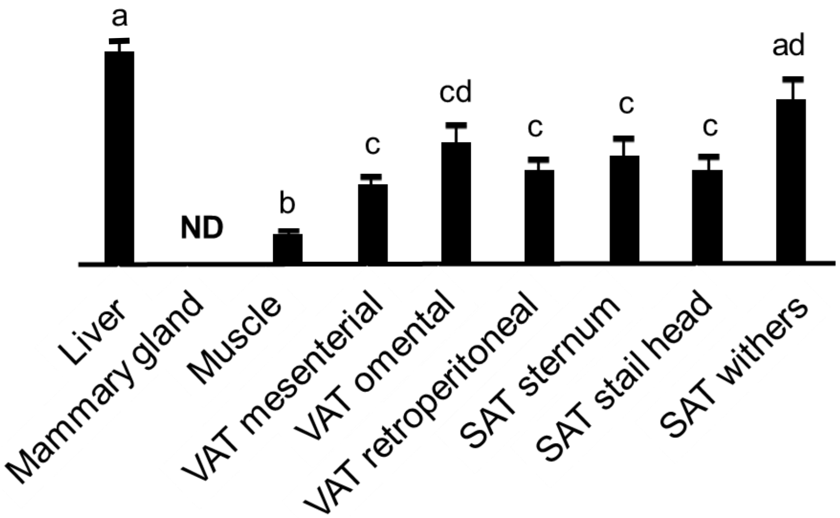


Hidalgo et al., 2011; Mena et al., 2013; Manosolva et al., 2015; Scalia et al., 2006

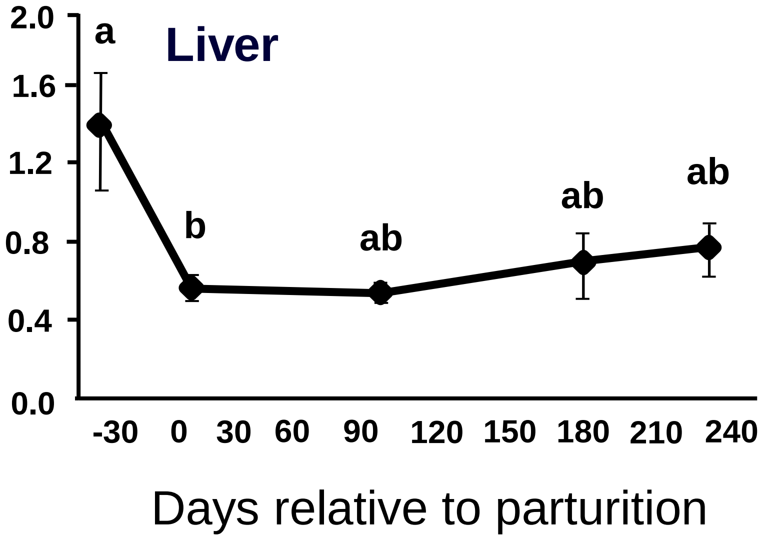
MMP-9: Matrix metalloprotease-9

FFAR1 mRNA in adipose tissue and liver of lactating dairy cows

Primiparous cow (slaughter)



Multiparous cow (biopsy)

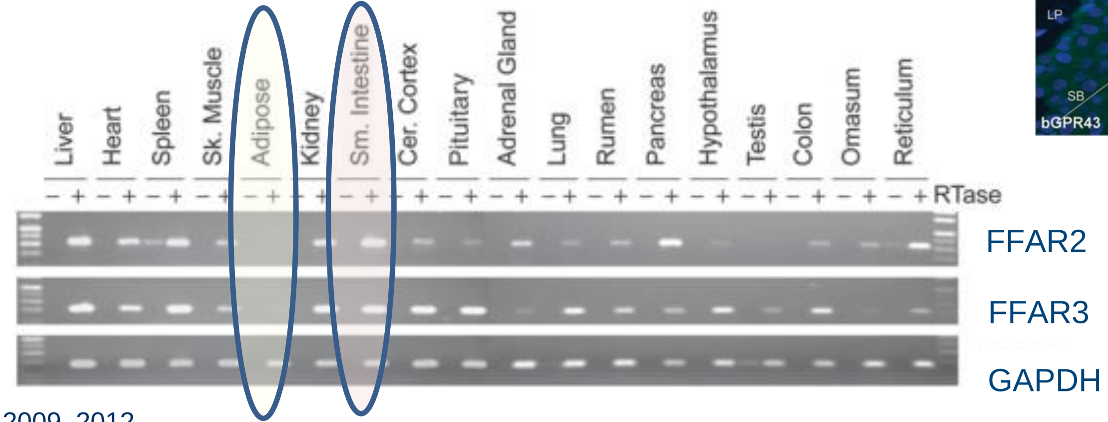


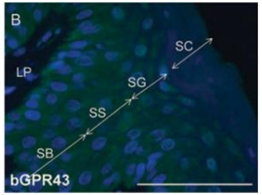
Friedrichs et al., 2014

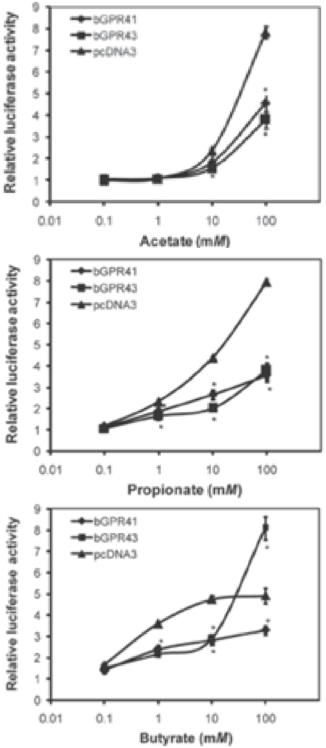
Family of free fatty acid binding receptors in ruminants

4

- Broad distribution of **FFAR2**, **FFAR3** mRNA abundance
- Cloning and functional analysis of bovine FFAR2 and FFAR3; two variants of FFAR3 mRNA
- FFAR2 protein detected in ruminal mucosa







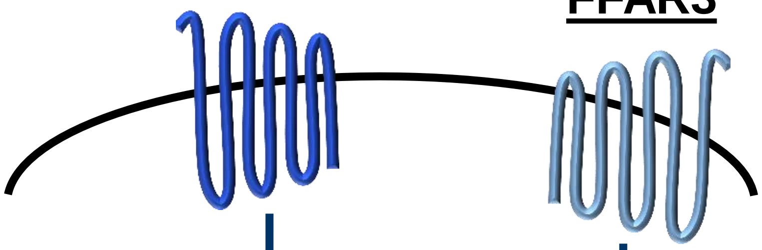
Wang et al., 2009, 2012

Differences in the affinity for SCFA of human compared to bovine FFAR2 and FFAR3

Human		Bovine	
Affinities:			
C3=C2>C1=C5	C3=C4=C5>C2>C1	C6>C5>C4>C3>C2	C5>C4<C3>C2

Affinity shifts to longer chain lenght of SCFA in the bovine

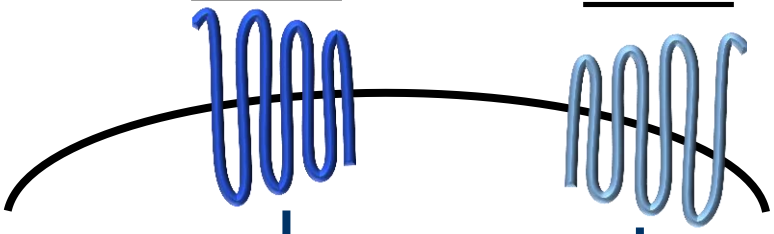
FFAR2 **FFAR3**



Gαq/11
Gαi/o

Gαi/o

FFAR2 **FFAR3**



Gαq/11
Gαi/o

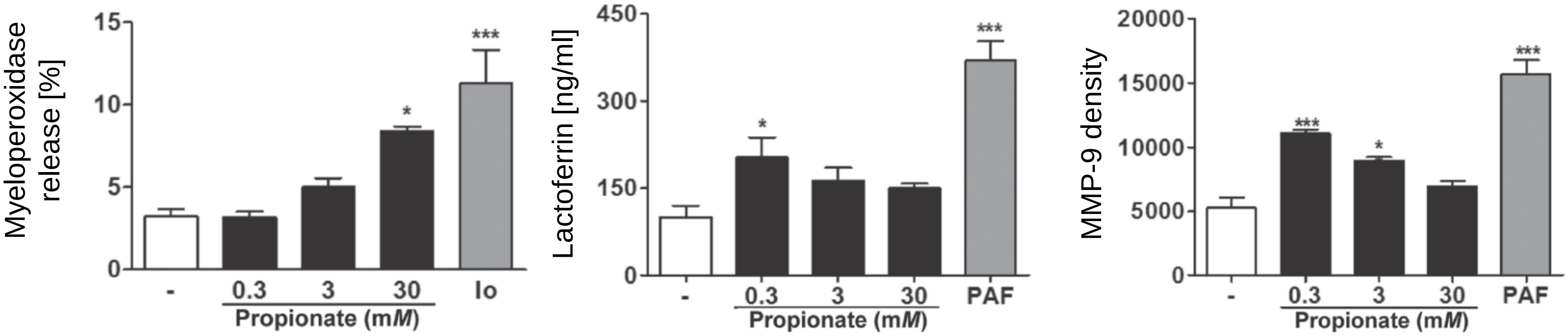
Gαi/o

Milligan et al., 2009
Hudson et al., 2012

Effects of SCFA on bovine neutrophils

4

- Propionate induces release of MMP-9, myeloperoxidase and lactoferrin



Caretta et al., 2013

Matrix metalloprotease-9 MMP-9

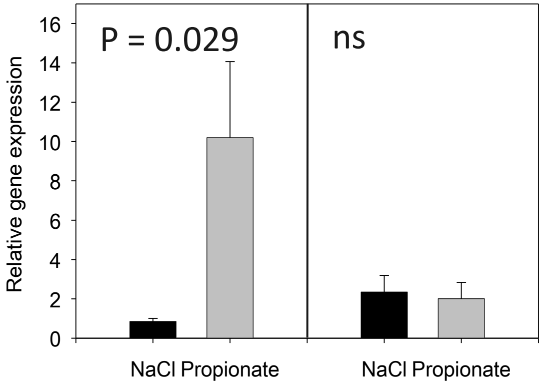
Effects of propionate on ruminant adipose tissue

- Infusion increases e.g. leptin and PPARγ mRNA in sheep
- Infusion or stimulation *in vitro* affects adipose tissue gene expression depot depending *in vivo* (goat) or *in vitro* (cow).

<u>Goat</u>

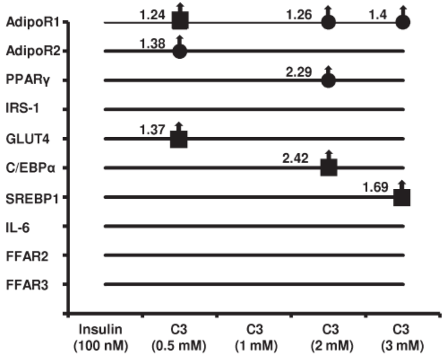
Subcutaneous Perirenal

FFAR3

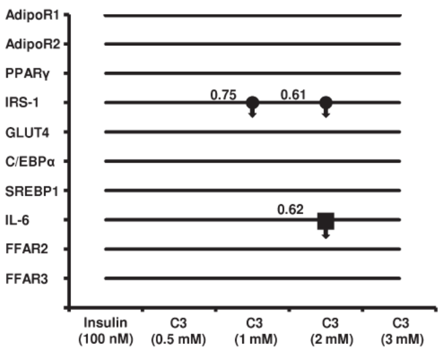


<u>Cow</u>

Subcutaneous



Kidney fat



● $P \leq 0.5$

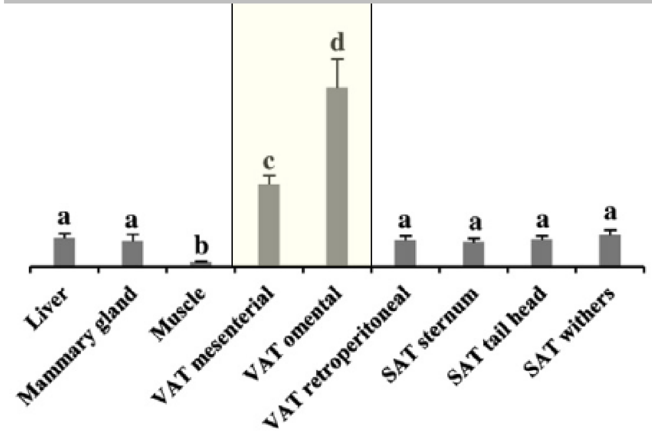
■ $P \leq 0.1$

Lee & Hossner, 2002; Hosseini et al., 2012; Mielenz et al., 2008

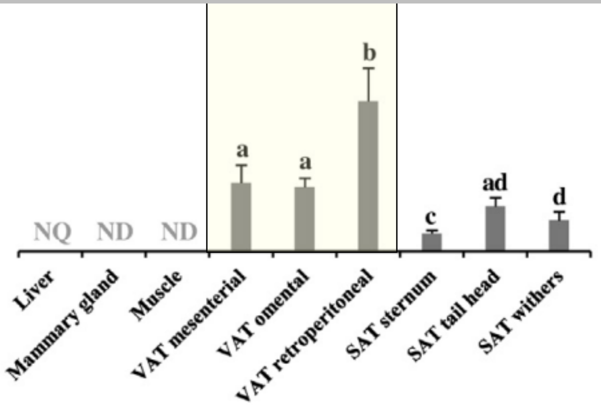
FFAR2 and FFAR3 mRNA in adipose tissue of dairy cow

- FFAR2 and FFAR3 with highest mRNA abundance in visceral adipose tissue depots of primiparous cows

FFAR2 mRNA – primiparous cows



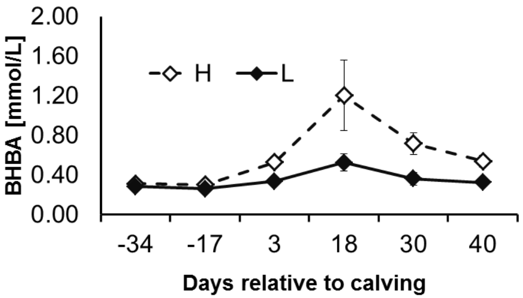
FFAR3 mRNA – primiparous cows

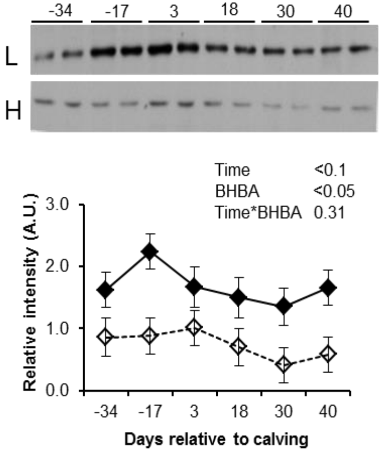


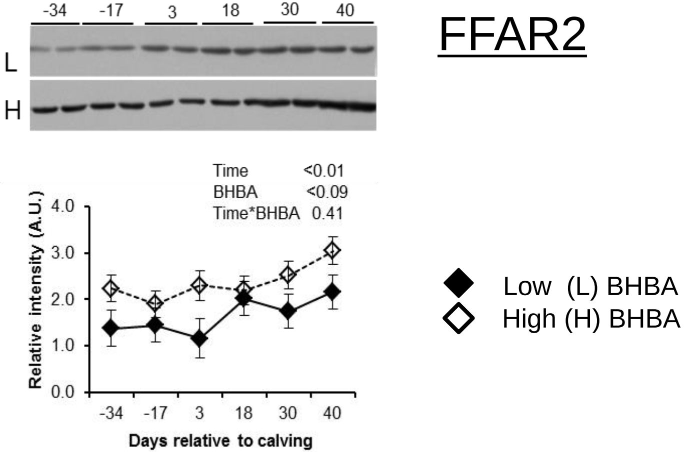
Friedrichs et al., 2014

FFAR1 and FFAR2 protein in liver of dairy cow during the transition period

- FFAR1 protein is differentially regulated depending on differences in lipid mobilisation (high BHBA vs. low BHBA in week 2 – 3 postpartum)
- FFAR2 protein is differentially regulated depending on time







Aguinaga et al., 2015 unpublished

4

Family of hydroxycarboxylic acid receptors in farm animals

Family of hydroxycarboxylic acid receptors in farm animals

4

- Currently no data on the receptors available for **chicken**

  → Effects of niacin and butyrate on lipid or insulin metabolism are shown.

  Jiang et al., 2014; Mátis et al., 2015

- Data on receptors in NCBI data base available for **sheep**

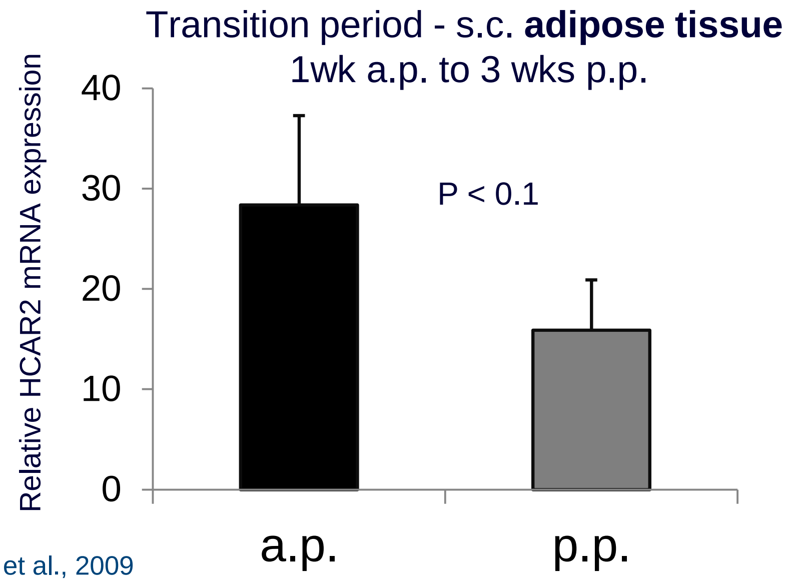
  → Niacin induces skeletal muscle fiber switch from type II to oxidative type I in **sheep**.

  Khan et al., 2013a, Khan et al., 2013b

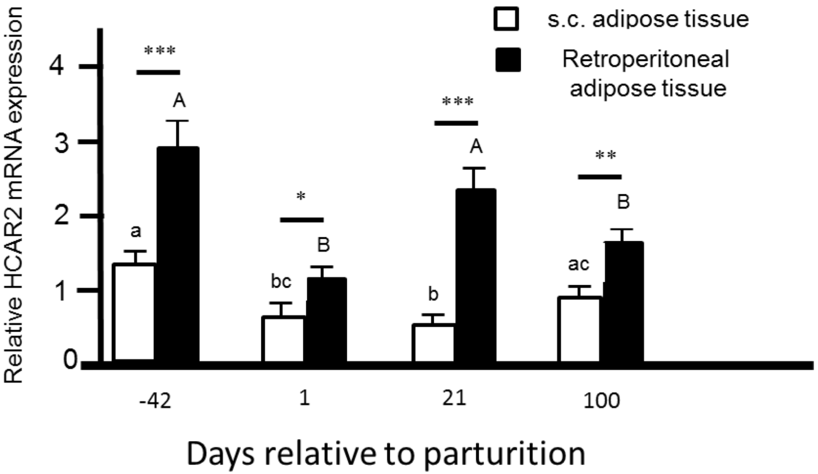
Family of hydroxycarboxylic acid receptors in cattle – HCAR2

- BHBA and butyrate reduces lipolysis in s.c. adipose tissue *in vitro*
- Inhibition of cAMP synthesis

Metz & van den Bergh, 1972; Metz, 1974



Lemor et al., 2009



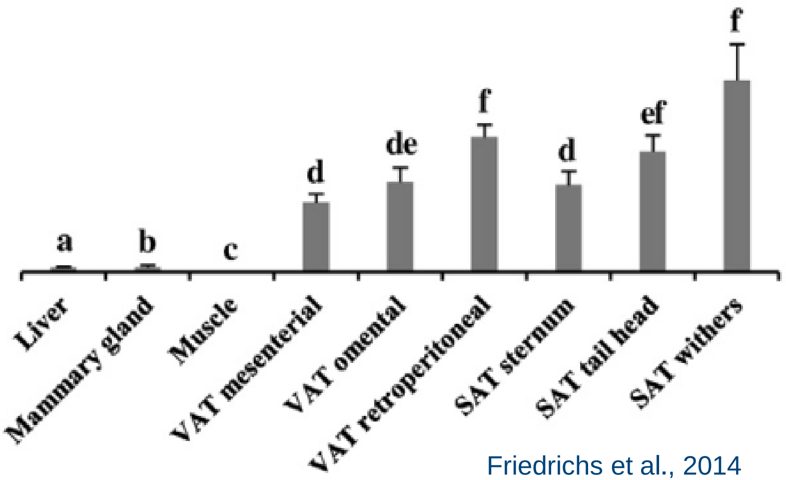
Weber et al., 2015, unpublished

Family of hydroxycarboxylic acid receptors in cattle – HCAR2

- BHBA and butyrate reduces lipolysis in s.c. adipose tissue *in vitro*
- Inhibition of cAMP synthesis

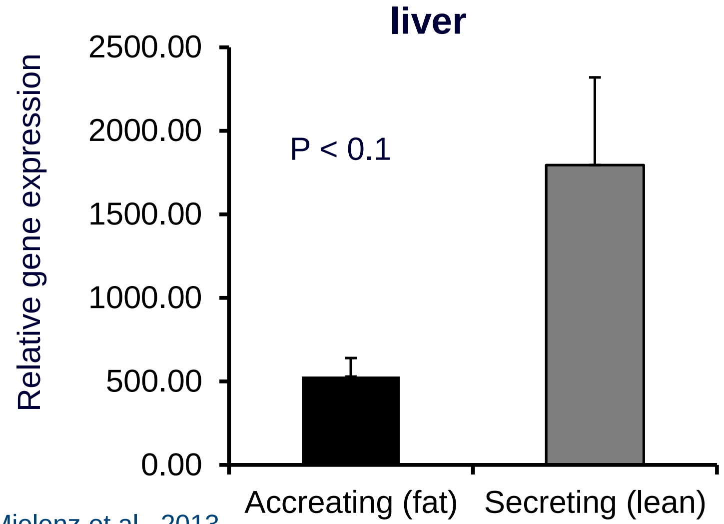
Metz & van den Bergh, 1972; Metz, 1974

HCAR2 mRNA – primiparous cows



Friedrichs et al., 2014

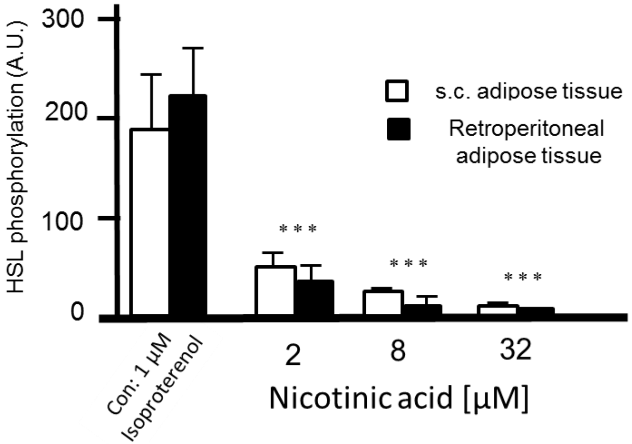
Charolais X German Holstein F2 offspring

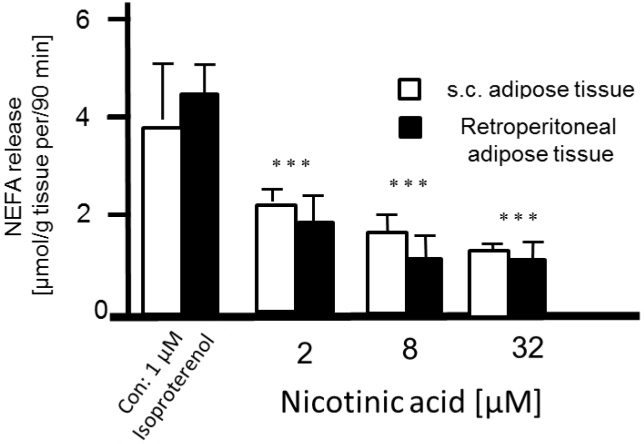


Mielenz et al., 2013

Family of hydroxycarboxylic acid receptors in cattle – HCAR2

- Nicotinic acid reduces the phosphorylation of hormone sensitive lipase (HSL).
- Nicotinic acid treatment reduces the stimulated NEFA release from bovine adipose tissue explants *in vitro*

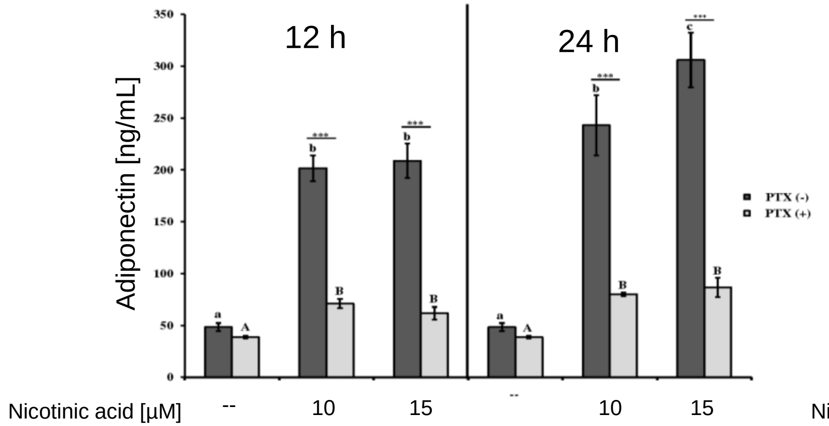


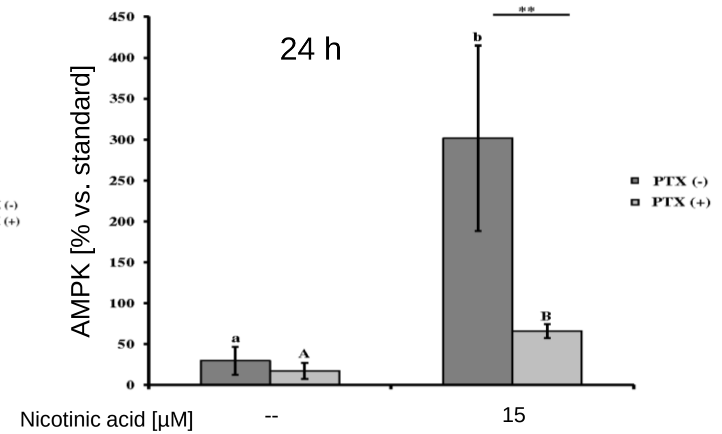


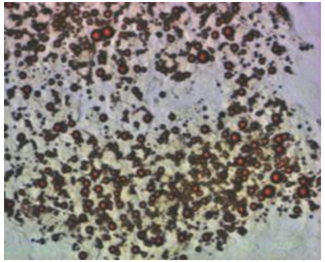
Kénez et al., 2014

Family of hydroxycarboxylic acid receptors in cattle – HCAR2

- Nicotinic acid increases adiponectin secretion, AMPK abundance and HCAR2 gene expression in differentiated bovine adipocytes *in vitro.*
- Nicotinic acid effects are partly Gi/o dependent (pertussis toxin (PTX) sensitive).



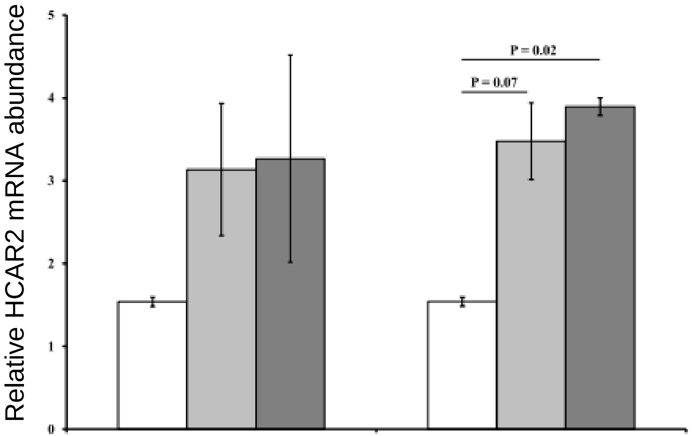


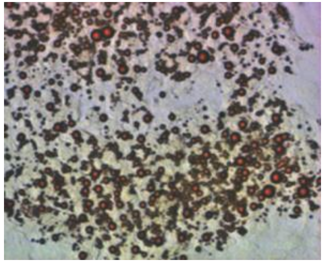


Kopp et al., 2014

Family of hydroxycarboxylic acid receptors in cattle – HCAR2

- Nicotinic acid increases HCA2 mRNA in differentiated bovine preadipocytes
- No effect of PTX

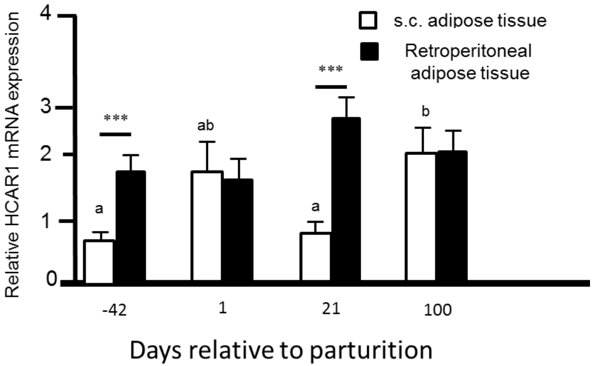




Kopp et al., 2014

Family of hydroxycarboxylic acid receptors in cattle – HCAR1

- HCAR1 mRNA in adipose tissue is reduced by high energy diet (1.62 Mcal/KG DM) vs. controlled energy diet (1.35 Mcal/KG DM) Ji et al., 2014
- Differential expression of HCAR1 mRNA during lactation
- Differential expression between s.c. vs. retroperitoneal adipose tissue



Weber et al., 2014

➡ Receptor binding of lactate characterised (EC_{50} 4.95 mM) Liu et al., 2009

- abomasal disorders
- exercise

➡ Autocrine lactate effects?

Conclusions

- Data on nutrient sensing receptors is currently mostly linked to the expression of receptor mRNA (protein data scarcely available)
- In many cases physiological significance has to be proven
- Relatively less knowledge on direct nutrient sensing-mechanisms

  Efeyan et al., 2015, Nature

  → especially in farm animals!

- Increasing knowledge may help to improve efficiency and health in farm animals in future

Acknowledgements

Institute of Nutritional Physiology "Oskar Kellner", FBN:

Maria Arantzazu Aguinaga Casañas

Harald Hammon
Björn Kuhla
Christine Schäff

Anne-Kathrin Möller

Institute of Muscle Biology and Growth, FBN:

Monika Röntgen

University of Veterinary Medicine Hannover

Clinic for Cattle:

Jürgen Rehage

Lena Locher

Department of Physiology

Korinna Huber*

Árkos Kénez*

Institute of Animal Science,
Physiology & Hygiene Unit,
University of Bonn:

Helga Sauerwein

Paula Friedrichs, Afshin Hosseini, Christina Kopp,
Anneka Lemor, Hamed Khalilvandi-Behroozyar,
Behnam Saremi, Shiva Singh, Martina Weber, Laura Webb

Inga Hofs
Isabella Israel

Institute of Animal Nutrition, Friedrich-Loeffler-Institute, Braunschweig
(FLI, Federal Research Institute for Animal Health)

Sven Dänicke
Ulrich Meyer
Dirk van Soosten
Reka Tienken

*present address: Institute of Animal Science, University of Hohenheim

LEIBNIZ INSTITUTE
FOR FARM ANIMAL BIOLOGY

How to find us

Leibniz-Institut für Nutztierbiologie FBN

Wilhelm-Stahl-Allee 2
18196 Dummerstorf

Contact

Manfred Mielenz

Phone: +49 38208 68 685
Fax: +49 38208 68 652
E-Mail: mielenz@fbn-dummerstorf.de
Internet: www.fbn-dummerstorf.de