Veterinärmedizinische Universität Wien

23. Tagung der Fachgruppe Physiologie und Biochemie der Deutschen Veterinärmedizinischen Gesellschaft

PROGRAMM & ABSTRACTS

21.02. – 23.02.2018

Tagungspräsident

Univ.-Prof. Dr.med.vet. Dr.med. Reinhold Erben
Institut für Physiologie, Pathophysiologie und Biophysik
Veterinärplatz 1, A-1210 Wien

Wissenschaftliches Organisationskomitee:

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Ao.Univ.-Prof. Dr.med.vet. Franz Schwarzenberger

Univ.-Prof. Dr.med. Elena Pohl

Univ.-Prof. Dr.sc.agr. Qendrim Zebeli

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Fachtagung DVG Physiologie und Biochemie ***21. – 23. Februar 2018***
Veterinärmedizinische Universität Wien, Austria

Liebe Kolleginnen und Kollegen,

ich begrüße Sie sehr herzlich zur 23. Tagung der DVG-Fachgruppe Physiologie und Biochemie an der Veterinärmedizinischen Universität Wien. Die im 2jährigen Rhythmus stattfindende Tagung der DVG-Fachgruppe Physiologie und Biochemie hat sich in den letzten Jahrzehnten zu einem wichtigen Medium des wissenschaftlichen Austausches in den Bereichen der Veterinär-Physiologie und Veterinär-Biochemie im deutschsprachigen Raum entwickelt. Es wird ja oft gesagt „Die Physiologie von heute ist die Medizin von morgen“. Dieser Satz unterstreicht die Wichtigkeit der Physiologie an der Schnittstelle zwischen den Grundlagenwissenschaften und der klinischen Medizin.

Wir hoffen, Ihnen ein interessantes wissenschaftliches Programm bieten zu können, das sowohl für die Wissenschaftlerinnen und Wissenschaftler aus den Grundlagenfächern als auch für Klinikerinnen und Kliniker Interessantes enthält. Für unsere beiden Keynote Lectures konnten wir sehr renommierte Sprecher gewinnen. Professor Stephen Bustin von der Anglia Ruskin University in UK, der als „Papst“ auf dem Gebiet der quantitativen PCR gilt, wird einen Vortrag halten zum Thema „Improving the analysis of quantitative PCR data in Veterinary research“. Die zweite Keynote Lecture mit dem Titel „Gut microbiota and metabolic health“ wird ERC-Preisträger Mirko Trajkovski von der Universität Genf halten. Die Einsicht, dass die Zusammensetzung des gastrointestinalen Mikrobioms entscheidenden Einfluss auf Stoffwechsel-Vorgänge im Wirt nehmen kann, wird in der nächsten Zukunft sicherlich viele Bereiche der Medizin revolutionieren. Insgesamt gesehen verspricht die Tagung einen sehr guten Überblick über die aktuelle Forschung im Bereich Veterinär-Biochemie und –Physiologie im deutschsprachigen Raum zu geben.

Ein Novum auf dieser Tagung sind die „Plenary Posters“, die beim Get-together am Eröffnungsabend und zusätzlich in einer eigenen Session als 5-Minuten-Kurzvorträge präsentiert werden. Die Auswahl als Plenary Poster beruht auf der Bewertung der Abstracts durch ein interdisziplinäres Team von Gutachterinnen und Gutachtern. Dabei wurden alle eingereichten Abstracts unabhängig von 3 – 4 Gutachtern bewertet. Als Plenary Posters wurden die 10 Abstracts mit der besten Bewertung ausgewählt, die als Poster eingereicht waren.

Für den Tagungsort Wien muss man kaum mehr Werbung machen. Im weltweiten Städte-Ranking hat Wien in den letzten Jahren stets auf dem ersten oder einem der ersten Plätze rangiert. Wien ist zu jeder Jahreszeit ein kulturelles und kulinarisches Highlight und immer eine Reise wert! Für den traditionellen Heurigenabend haben wir einen Wirt für Sie ausgesucht, der zum besten Heurigenwirt 2016 in ganz Österreich gekürt wurde.

Ich freue mich gemeinsam mit Ihnen auf eine interessante Tagung!

Reinhold Erben

Univ.-Prof. DDr. Reinhold G. Erben

Fachtagung DVG Physiologie und Biochemie ***21. – 23. Februar 2018***
Veterinärmedizinische Universität Wien, Austria

PROGRAMM

Mittwoch, 21.02.2018	
15:00 - 17:00 Uhr	**Workshop „PCR assay design“ mit Stephen A. Bustin**, Anglia Ruskin University, UK
16:00 - 17:00 Uhr	**Hochschullehrerversammlung**
17:30 - 19:00 Uhr	**Welcome Reception, Plenary Posters & Ausstellung**
19:00 - 20:00 Uhr **K01**	**Eröffnung und Keynote Lecture** Vorsitz: Reinhold Erben, Herbert Fuhrmann **Stephen A. Bustin**, Anglia Ruskin University, UK ***Improving the analysis of quantitative PCR data in Veterinary research***
Donnerstag, 22.02.2018	
Session [08:30 - 10:00 Uhr] **Epitheliale Barriere und Mikrobiom-Stoffwechsel-Interaktionen** Vorsitz: Teresa Valencak, Qendrim Zebeli	
08:30 - 09:30 Uhr **K02**	**Keynote Lecture** **Mirko Trajkovski, Universität Genf** ***Gut microbiota and metabolic health***
09:30 - 10:00 Uhr **V01** **V02**	**Vorträge (je 10 Min. + 5 Min. Diskussion)** **Functional role of nicotinic receptors in the colon** Lena Lottig, Martin Diener, Gießen **Inhibition of the non-neuronal cholinergic system alters the expression of tight junction proteins in PoCo83-3 cells** Bastian Kaiser, Johannes Kacza, Gotthold Gäbel, Helga Pfannkuche, Leipzig
10:00 - 10:30 Uhr	**Kaffeepause und Ausstellung**

Session [10:30 - 12:30 Uhr]
Stoffwechselphysiologie und Regeneration
Vorsitz: Ralf Einspanier, Cornelia Kasper

10:30 - 11:00 Uhr	**Teresa Valencak**, Veterinärmedizinische Universität Wien
Ü01	***Energy Metabolism & Lactation in Rodents***
11:00 - 12:30 Uhr	**Vorträge (je 10 Min. + 5 Min. Diskussion)**
V03	**Real time breath analysis of volatile organic compounds in bovines** Anne Küntzel, Peter Oertel, Phillip Trefz, Wolfram Miekisch, Jochen Schubert, Heike Köhler, Petra Reinhold, Jena / Rostock
V04	**Influence of dietary nitrogen on the hepatic signalling pathway of the somatotropic axis in young goats** Caroline Firmenich, Nadine Schnepel, Alexandra Muscher-Banse, Hannover
V05	**Effect of short chain fatty acids and ß-hydroxybutyrate on gene expression of gluconeogenic enzymes in the bovine liver cell line BFH12** Anna-Maria Sittel, Herbert Fuhrmann, Axel Schoeniger, Leipzig
V06	**Dorsal shaving improves milk energy output and lowers heat stress in golden hamster females (Mesocricetus auratus)** Sarah Ohrnberger, Rupert Palme, Teresa Valencak, Wien
V07	**BET protein family member BRD4 promotes transcription through super enhancer dynamics in kidney repair and progression of fibrosis** Julia Wilflingseder, Michaela Willi, Chaochen Wang, Hannes Olauson, Takaharu Ichimura, Todd Valerius, Lothar Hennighausen, Joseph Bonventre, Wien / Bethesda / Stockholm / Boston
V08	**Context-sensitive immunomodulation by equine multipotent mesenchymal stromal cells in a novel co-culture assay** Janina Burk, Felicitas Päbst, Walter Brehm, Daniel Piehler, Susanna Schubert, Attila Tárnok, Aline Hillmann, Wien / Naharya / Leipzig
12:30 - 13:30 Uhr	**Mittagspause**
13:30 - 14:30 Uhr	**Posters und Ausstellung**

Session [14:30 -16:00 Uhr]
Immunologie, Neuroimmunologie und Neurophysiologie I
Vorsitz: Maren von Köckritz-Blickwede / Bernd Kaspers

14:30 - 15:00 Uhr	**Christoph Rummel, Justus-Liebig-Universität Gießen**
Ü02	***Inflammatory mediators and transcription factors: brain-controlled components of the acute phase response***

15:00 – 16:00 Uhr	**Vorträge (je 10 Min. + 5 Min. Diskussion)**
V09	**Age dependent hypothalamic and pituitary responses to novel environment stress or lipopolysaccharide in rats** Christoph Rummel, Sandy Koenig, Janne Bredehöft, Alexander Perniss, Franziska Fuchs, Joachim Roth, Gießen
V10	**Different immune capacities of cows after polyclonal immune stimulation** Karina Lutterberg, Bernhard Hobmaier, Kristina Kleinwort, Stefanie Hauck, Cornelia Deeg, München
V11	**Equine recurrent uveitis - Protein expression differences in effector cells** Roxane Degroote, Kerstin Euler, Stefanie Hauck, Cornelia Deeg, München
V12	**Protein-protein-interaction of septin 7 and DOCK8 unravels pathomechanisms in ERU** Melanie Schauer, Roxane Degroote, Stefanie Hauck, Carmen Wiedemann, Elisabeth Kremmer, Cornelia Deeg, München
16:00 - 16:30 Uhr	**Kaffeepause und Ausstellung**

Session [16:30 -17:30 Uhr]
Immunologie, Neuroimmunologie und Neurophysiologie II
Vorsitz: Cornelia Deeg / Christoph Rummel

16:30 – 17:30 Uhr	**Vorträge (je 10 Min. + 5 Min. Diskussion)**
V13	**Comparison of neutrophil extracellular trap formation in response to Trypanosoma cruzi infection in dogs and opossums** Nicole de Buhr, Marta Cristina Bonilla, Mauricio Jimenez-Soto, Gaby Dolz, Maren von Köckritz-Blickwede, Hannover / Costa Rica
V14	**Effect of hypoxia on innate immune cell function: Cell type specific effects comparing neutrophils and mast cells** Maren von Köckritz-Blickwede, Katja Branitzki-Heinemann, Helene Möllerherm, Graham Brogden, Herbert Fuhrmann, Hassan Y. Naim, Hannover / Leipzig

V15	**Primary culture of the rat substantia gelatinosa as a tool to investigate the effects of inflammatory stimulation on the afferent somatosensory system** Stephan Leisengang, Daniela Ott, Jolanta Murgott, Rüdiger Gerstberger, Christoph Rummel, Joachim Roth, Gießen
V16	**Existence of a local renin-angiotensin system in the hypothalamic Organum subfornicale: functional characterization of a SFO-specific primary cell culture system** Niklas Grabbe, Rüdiger Gerstberger, Gießen

DVG-Mitgliederversammlung [17:30 - 18:00 Uhr]

Wiener Heurigen-Abend [19:30 – 22:30 Uhr] (**nur mit Ticket!**)
Heuriger Wieninger, Stammersdorfer Straße 78, 1210 Wien

Freitag, 23.02.2018

Session [09:00 - 10:30 Uhr]
Epithel, epitheliale Barriere und Transportphysiologie I
Vorsitz: Martin Diener, Helga Pfannkuche

09:00 – 10:30 Uhr **Vorträge (je 10 Min. + 5 Min. Diskussion)**

V17	**Investigation of the NH4+ conductance of the human analogue of TRPV3** Hendrik Liebe, Franziska Liebe, Constanze Vitzthum, Gerhard Sponder, Friederike Stumpff, Berlin
V18	**Butyrate – regulator of pathways in ovine ruminal epithelium?** Lisa Baaske, Gotthold Gäbel, Leipzig
V19	**Effects of the absorption enhancer caprate on follicle-associated epithelium of porcine Peyer's patches** Judith Radloff, Valeria Cornelius, Evgeny Falchuk, Alexander Markov, Salah Amasheh, Berlin / St. Petersburg
V20	**Hypoxia in jejunum epithelium: fast adaptation of glucose transport** Franziska Dengler, Reiko Rackwitz, Helga Pfannkuche, Gotthold Gäbel, Leipzig
V21	**Effects of feeding graded levels of deoxynivalenol and oral administration of lipopolysaccharide on expression of barrier function and innate immune genes in the small intestine of chickens** Annegret Lucke, Josef Böhm, Qendrim Zebeli, Barbara Metzler-Zebeli, Wien

V22	**Permeation of butyrate across the ovine reticulum epithelium** Reiko Rackwitz, Gotthold Gäbel, Leipzig
10:30 - 11:00 Uhr	**Kaffeepause und Ausstellung**
Session [11:00 - 12:30 Uhr] **Oral Posters** Vorsitz: Julia Wilflingseder / Christoph Rummel	
11:00 – 12:30 Uhr	**Vorträge (je 5 Min. + 3 Min. Diskussion)**
P01	**The bovine rumen expresses bTRPV3 channels as a pathway for the uptake of NH4+** Franziska Liebe, Hendrik Liebe, Constanze Vitzthum, Sabine Käßmeyer, Gerhard Sponder, Friederike Stumpff, Berlin
P02	**Safety and migration of intra-articularly injected mesenchymal stem cells in a focal cartilage defect model** Maria Satue, Reinhold Erben, Wien
P03	**Characterization of chicken TNF-a, a long missed cytokine in birds** Franziska Rohde, Benjamin Schusser, Tomáš Hron, Helena Farkašová, Jiri Plachý, Jiri Hejnar, Daniel Elleder, Bernd Kaspers, München / Prag
P04	**Butyrate - guardian of the porcine colon epithelium?** Anika Kraetzig, Gotthold Gäbel, Franziska Dengler, Leipzig
P05	**Pre-enrichment of Mycobacterium avium subsp. paratuberculosis using plant lectins** Bernhard Hobmaier, Karina Lutterberg, Kristina Kleinwort, Ricarda Mayer, Sieglinde Hirmer, Florian Gosselin, Cornelia Deeg, München
P06	**Endothelium-Specific Vitamin D Receptor Ablation Fails to Recapitulate the Cardiovascular Phenotype of Global Vitamin D Receptor Knockout Mice** Nejla Latic, Svetlana Slavic, Olena Andrukhova, Ute Zeitz, Geert Carmeliet, Reinhold Erben, Wien / Leuven
P07	**Gel-based proteomics to learn more about effects of the flame retardant HBCD on the liver proteome of female rats** Ingrid Miller, Tommaso Serchi, Jenny Renaut, Tinka Murk, Arno Gutleb, Wien / Wageningen / Luxembourg

P08	**Isolation of exosomes from blood serum and cell culture supernatants for diagnostic purposes in dogs** Ralf Einspanier, Matias Aguilera-Rojas, Barbara Kohn, Johanna Plendl, Berlin
P09	**The role of DOCK8 and its interaction partners in lymphocytes of ERU cases** Carmen Wiedemann, Melanie Schauer, Roxane Degroote, Barbara Amann, Stefanie Hauck, Cornelia Deeg, München
P10	**Effects of bilateral hydrostatic pressure incubation on the barrier function of mammary epithelial cells** Katharina Mießler, Alexander Markov, Salah Amasheh, Berlin / St. Petersburg
12:30 - 13:30 Uhr	**Mittagspause**
13:30 - 14:30 Uhr	**Posters und Ausstellung**

Session [14:30 -16:00 Uhr]
Epithel, epitheliale Barriere und Transportphysiologie II
Vorsitz: Rainer Cermak, Jörg Aschenbach

14:30 – 16:00 Uhr	**Vorträge (je 10 Min. + 5 Min. Diskussion)**
V23	**Dietary methionine source influences the expression profile of methionine transport systems in the gastrointestinal tract of growing pigs** Lucia Mastrototaro, Stella Romanet, Robert Pieper, Jürgen Zentek, Behnam Saremi, John Htoo, Jörg Aschenbach, Berlin
V24	**Impact of inflammation on neuro-immune interactions in the intestine of rats** Jasmin Becker, Martin Diener, Gießen
V25	**Influence of dietary menthol-based bioactive lipid compounds on conductance and uptakes of glucose and methionine in the rumen and intestine of sheep** Amlan Patra, Sebastian Geiger, Hannah-Sophie Braun, Jörg Aschenbach, Berlin
V26	**Campylobacter jejuni modulates the intestinal mucosal barrier function with consequences on bacterial translocation in chickens** Wageha Awad, Claudia Hess, Jörg Aschenbach, Károly Dublecz, Michael Hess, Wien / Keszthely

V27	**Effects of lactic acid treatment of cereals and dietary phytase on phosphorus digestibility and bone parameters in growing pigs** Julia Vötterl, Jutamat Klinsoda, Qendrim Zebeli, Barbara Metzler-Zebeli, Wien
V28	**A four-week high-concentrate feeding alters hindgut microbiome leading to dysbiosis in dairy cows** Viktoria Neubauer, Renee Petri, Iris Kröger, Elke Humer, Nicole Reisinger, Qendrim Zebeli, Wien / Tulln
16:00 - 16:30 Uhr	**Kaffeepause und Ausstellung**
Session [16:30 - 17:45 Uhr] **Freie Themen** Vorsitz: Gotthold Gäbel, Elena Pohl	
16:30 - 17:00 Uhr	**Reinhold Erben, Veterinärmedizinische Universität Wien**
Ü03	***FGF23: The good, the bad, and the ugly***
17:00 – 17:45 Uhr	**Vorträge (je 10 Min. + 5 Min. Diskussion)**
V29	**Evaluating the biological sensitivity of non-invasive methods for measuring adrenocortical activity: an example in cattle** Rupert Palme, Florian Wetscher, Wien
V30	**The hepatocyte-derived cell line BFH12 as a new in vitro model for bovine biotransformation** Axel Schöniger, Lydia Kuhnert, Herbert Fuhrmann, Leipzig
V31	**Comparative analysis of gestation in three rhinoceros species (Diceros bicornis, Ceratotherium simum, Rhinoceros unicornis)** Franz Schwarzenberger, Wien
Schlussbetrachtung und Verabschiedung [17:45 - 18:00 Uhr]	

KEYNOTE LECTURES

K01
Improving the analysis of quantitative PCR data in Veterinary research

Stephen A. Bustin, Anglia Ruskin University, UK

The emergence of molecular veterinary medicine has been driven by the availability of sequence information on pathogens and species relevant for economic (e.g. salmon, chicken, pig, cattle) and social (e.g. horse, dog, cat) purposes. The various areas of veterinary research are making use of a wide range of molecular tools, resulting in the description of animal diseases in molecular terms, both at the genetic and at the functional genomics levels. The most widely used molecular technique is the polymerase chain reaction (PCR), and quantitative reverse transcription PCR (RT-qPCR) in particular, which is extensively applied to the quantification of differential gene expression patterns associated with various experimental and/or clinical conditions. The increasing awareness of significant problems associated with this technology resulted in 2009 in the publication of the MIQE guidelines, which aim to guide researchers with respect to assay design and transparent publication of data. The objectives of this presentation are to highlight the challenges apparent in the molecular veterinary medicine literature and to provide solutions to encourage the publication of biologically meaningful molecular data in this important and expanding area.

K02
Gut microbiota and metabolic health

Mirko Trajkovski, Universität Genf, Schweiz

Food intake, energy expenditure and body adiposity are homeostatically regulated, and malfunctions of this balance can cause obesity. Brown adipose tissue (BAT) catabolizes calories to produce heat, and its function can be induced by prolonged cold exposure and beta-adrenergic stimulation. BAT is present at distinct anatomical sites, including the interscapular, perirenal, and axillary depots. In response to cold or caloric restriction, brown fat cells also emerge in subcutaneous white fat (known as “beige” cells), a process referred to as WAT browning. Increased beige and brown fat development promote energy expenditure and improve insulin sensitivity, suggesting the manipulation of the fat stores as an anti-obesity therapeutic perspective.

The intestinal microbiota co-develops with the host, and its composition is influenced by several physiological, pathological and environmental factors. The microbiota influences the whole-body metabolism by affecting the energy balance on multiple levels. Cold exposure leads to marked shift of the microbiota composition, and transplantation of this cold-altered microbiota to germ-free mice is sufficient to increase insulin sensitivity of the host and enable tolerance to cold partly by promoting brown fat development and inducing white fat browning, leading to increased energy expenditure and fat loss. During prolonged cold however, the body weight loss is attenuated, caused by adaptive mechanisms maximizing caloric uptake and increasing intestinal, villi, and microvilli lengths. This increased absorptive surface is transferable with the cold microbiota, leading to altered intestinal gene expression promoting tissue remodeling and suppression of apoptosis. I will discuss our recent findings that suggest molecular explanation of the microbiota signaling to the host in regulating the energy homeostasis.

ÜBERSICHTS-VORTRÄGE

Ü01
Energy Metabolism & Lactation in Rodents

Teresa Valencak, Veterinärmedizinische Universität Wien, Austria

When energy intake and energy expenditure are balanced, body weight does not change but remains constant. In lactating rodents however, body weight even increases during lactation although energy expenditure largely outweighs energy or food intake during the commonly three weeks long lactation period. Not only do females massively increase their food intake but also all organs work at peak rates. What are the consequences of peak metabolic rates over a long time? What may represent a limit to energy intake even when exposed to ad libitum food availability conditions? In my talk, I will provide an overview on previous experimental studies in mice, Mongolian gerbils and golden hamsters by emphasising the role of metabolic heat during the process of lactation. When raising offspring, females are observed to show higher body temperatures, a thinner fur and finally, down-regulated expression of uncoupling protein 1 (UCP-1). I will also discuss future potential applications of these observations in context with breeding laboratory rodents for their use in contemporary biomedical research.

Ü02

Inflammatory mediators and transcription factors: brain-controlled components of the acute phase response

Christoph Rummel, Justus-Liebig-Universität Gießen, Deutschland

Brain-controlled components of the acute phase response include fever, anorexia, adipsia, and social isolation. These changes originate from the action of the innate immune system on the brain. Underlying immune-to-brain communication pathways involve humoral mediators including cytokines, central modulation via neuronal afferents and immune cell trafficking to the brain. During systemic inflammation, these pathways contribute to mediating the brain-controlled sickness symptoms like fever. Experimentally, activation of these signaling pathways can be mimicked and studied when injecting animals with pathogen associated molecular patterns (PAMPS; i.e. models for viral or bacterial infection). One central component of the brain inflammatory response, which leads, for example, to fever induction, is transcriptional activation of brain cells via cytokines and PAMPS like lipopolysaccharide (LPS). We and others have studied the spatiotemporal activation and the physiological significance of transcription factors for the induction of inflammation within the brain and the manifestation of fever during systemic inflammation induced by various PAMPS. Evidence has revealed a role of nuclear factor (NF)κB in the initiation, signal transducer and activator of transcription (STAT)3 in the maintenance and NF-interleukin (IL)6 in the maintenance or even termination of brain-inflammation and fever. As an anti-inflammatory strategy, we have tested several inhibitors of transcription factor activation. Indeed, numerous exogenous natural compounds like parthenolide as well as endogenous mediators such as glucocorticosteroids do inhibit transcription factor activation. Recently, we also tested the potential of vagus nerve stimulation to treat early septic-like inflammation and to alter transcription factor activation in the brain. Indeed, this treatment stabilized neuro-vascular coupling and reduced NF-IL6-activation in the brain during septic-like inflammation. Interestingly, the nutritional status, as reflected by circulating levels of the cytokine-like hormone leptin, influence immune-to-brain communication and age-dependent changes in LPS-induced fever and plasma leptin concentration can be reduced by vagus nerve stimulation. In summary, we gained new insights into immune-to-brain communication and its modulation, for instance, by the nutritional status and aging with the use of inflammatory transcription factors as activation markers in the brain. Moreover, inflammatory transcription factors remain therapeutically important targets for the treatment of brain-inflammation and fever induction during infectious / non-infectious inflammatory stress.

Ü03
FGF23: The good, the bad, and the ugly

Reinhold Erben, Veterinärmedizinische Universität Wien, Austria

Fibroblast growth factor-23 (FGF23) is a bone-derived hormone, mainly produced by osteoblasts and osteocytes in response to increased extracellular phosphate and circulating vitamin D hormone. Under physiological conditions, endocrine FGF23 signaling requires co-expression of the ubiquitously expressed fibroblast growth factor receptor 1c (FGFR1c) and the co-receptor α-Klotho (Klotho). In proximal renal tubules, FGF23 suppresses the membrane expression of the type II sodium-phosphate cotransporters Npt2a and Npt2c which mediate urinary re-absorption of filtered phosphate. In addition, FGF23 suppresses proximal tubular expression of 1α-hydroxylase, the key enzyme responsible for vitamin D hormone production. Therefore, FGF23 protects against hyperphosphatemia by directly increasing urinary phosphate excretion und indirectly decreasing vitamin D hormone-dependent intestinal phosphate absorption. However, FGF23 not only acts on proximal renal tubules, but also targets distal renal tubules, where FGF23 signaling activates with-no-lysine kinase 4, leading to increased renal tubular re-absorption of calcium and sodium. Therefore, FGF23 is not only a phosphaturic, but also a calcium- and sodium-conserving hormone involved in blood pressure regulation, a finding which may have important implications for the pathophysiology of chronic kidney disease (CKD). Moreover, in diseases characterized by increased endogenous secretion of FGF23 such as CKD, Klotho-independent signaling mechanisms may become important in various organ systems. In this context, it was shown that high circulating levels of FGF23 may lead to heart hypertrophy via a FGFR4-mediated, Klotho-independent signaling cascade. In addition, we recently found that excessive FGF23 signaling is a pro-inflammatory factor in the kidney, directly acting on renal epithelial cells to promote inflammation and fibrosis in a Klotho-independent manner. Hence, excessive FGF23 signaling may have an important role as a disease-driving factor in the pathophysiology of acute and chronic kidney disease.

VORTRÄGE

Fachtagung DVG Physiologie und Biochemie ***21. – 23. Februar 2018***
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V01
Functional role of nicotinic receptors in the colon

Lottig, Lena (Institut für Veterinärphysiologie, Uni Giessen, Gießen, GER); Diener, Martin (Institut für Veterinärphysiologie, Uni Giessen, Giessen, GER)

One of the most important physiological regulators of colonic functions is acetylcholine. Acetylcholine is released from cholinergic neurons from where it diffuses to two types of receptors, i.e. nicotinic and muscarinic receptors. In the peripherous nervous system, nicotinic receptors are in generally thought to mediate the effect of acetylcholine in excitable tissue such as neurons or skeletal muscle. The view on the colinergic system was revolutionized when the synthesis of acetylcholine was detected in non-neuronal cells and nicotinic receptors were found to be expressed by non-excitable tissue, too.

To investigate the role and the potential signal mechanism of epithelial nicotinic receptors in the large intestine, Ussing chamber experiments were performed using mucosa-submucosa preparations from rat distal colon. Stimulation of non-neuronal nicotinic acetylcholine receptors (nAChR) in this tissue induces a chloride secretion, which is a physiological function of the epithelium resulting in the secretion of water. Apically permeabilized epithelia were used to selectively measure currents across the basolateral membrane. These experiments showed that nicotine acts via stimulation of the Na^+/K^+-pump.

Changes in intracellular Na^+ concentration were monitored in imaging experiments using the sodium-sensitive dye SBFI on isolated crypts. A decrease of the cytosolic Na^+ concentration was observed after addition of nicotine, supporting the idea of a stimulation of the pump current via nAChR. This effect was independent of the presence of Ca^{2+} in the solution. These results refute the possibility of Na^+ or Ca^{2+} influx through nACHR and suggest that the nAChR in the colonic epithelium differs from the subtypes in the central nervous system, where this receptors acts as a classical ionotropic receptor, i.e. as a non-selective, ligand-gated cation channel. Based on inhibition experiments we found that a protein kinase as well as a G protein may be involved in the signal transduction. Thus it is like that nAChR in the colonic epithelium acts as a metabotropic receptor.

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V02

Inhibition of the non-neuronal cholinergic system alters the expression of tight junction proteins in PoCo83-3 cells

Kaiser, Bastian (Uni-Leipzig, Leipzig, GER); Kacza, Johannes (Uni-Leipzig, Leipzig, GER); Gäbel, Gotthold (Uni-Leipzig, Leipzig, GER); Pfannkuche, Helga (Uni Leipzig, Leipzig, GER)

Non-neuronal acetylcholine is expressed in various cell types including epithelial cells. It functions as a modulator of basic cell properties probably including modulation of barrier functions of the epithelial layer. In the present study, we aimed to investigate whether inhibition of acetylcholine expression or blockage of cholinergic receptors alter integrity of the epithelial barrier in the porcine colonic cell line PoCo83-3.

PoCo83-3 cells were cultured on membrane filter supports (for measurement of transepithelial electrical resistance (TEER) and real-time PCR) or on chamber slides (for confocal laser scanning microscopy) and treated either with the choline acetyltransferase (ChAT) inhibitor bromoacetylcholine (BrACh), the muscarinic antagonist atropine or the nicotinic antagonist mecamylamine (MEC). All these treatments resulted in a significant decrease in TEER of the cell layer compared to control conditions. In contrast, the muscarinic antagonist 4-diphenylacetoxy-N-methylpiperidine methiodide (4-DAMP) and the nicotinic antagonists a-bungarotoxin and tubocurarine only had a negligible effect on TEER.

LDH release assays showed that the observed effects on TEER were not due to cytotoxic effects of the active compounds as the LDH release from treated cells did not significantly differ from the control.

Real-time PCR revealed alterations in tight junction-related gene expression. We observed a significant downregulation of claudin 3 gene expression in cells exposed to BrACh for 12 h, while claudin 1 (CLDN1) and occludin gene expression was upregulated. Zonula occludens 1 (ZO-1) mRNA expression was downregulated in cells exposed to BrACh or atropine treated for 12 h.

Immunofluorescent staining in combination with confocal laser scanning microscopy showed an increase in CLDN1 protein signal intensity in BrACh treated cells compared to the control after 48 h. Additionally, in some specimen treated with atropine or MEC the staining pattern of ZO-1 was altered in combination with the loss of CLDN1 expression.

We suggest PoCo83-3 as a suitable model to investigate the role of the non-neuronal ACh in regulating barrier function of the porcine colonic epithelium. A dysfunctional non-neuronal cholinergic system leads to alterations in epithelial barrier integrity probably by altering tight junction composition.

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V03
Real-time breath analysis of volatile organic compounds in bovines

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Background: In human and veterinary medicine, the search for exhaled volatile organic compounds (VOC) as potential markers for disease detection has gained increasing attraction because exhaled breath analysis bears the advantages of being continuous, non-invasive and presenting instantly available results. The aim of this study was to introduce a new technique for real-time breath analysis in bovines.

Animals and Methods: The technical set-up enabled (i) CO_2-controlled sampling of alveolar gas and (ii) direct breath gas analysis via proton transfer reaction–time of flight–mass spectrometry (PTR-ToF-MS) in parallel. While alveolar gas sampling enabled VOC analysis by gas chromatography-mass spectrometry for unequivocal substance identification, PTR-ToF-MS ensured real-time identification of confounding signals within one measurement. Two male bovines aged 5–6 months were housed under standardized conditions. In each animal, breath analysis was carried out 6 times with two hour intervals throughout the day, and in duplicate on two separate days.

Results: The new system for real-time breath analysis was successfully applied to adolescent cattle. Proof-of principle measurements revealed significant effects of daytime on VOC composition. Habituation to the procedure and training of the animals stabilized measurements results. All factors influencing biological variability need to be evaluated in-depth by future studies in larger groups of animals.

Conclusions: Breath gas analysis opens an interesting research area in veterinary medicine. (1) From a clinical perspective, it could promote the search for new biomarkers of health and disease. (2) In the experimental field, real-time breath analysis is one step towards refinement of large animal studies.

V04

Influence of dietary nitrogen on the hepatic signalling pathway of the somatotropic axis in young goats

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Ruminants are thought to cope easily with a reduced nitrogen (N) intake due to efficient rumino-hepatic circulation of urea. However, in previous studies it was found that decreased dietary N intake caused massive changes in mineral homeostasis in young goats (1, 2). During an N-reduced diet, blood calcium (Ca), calcitriol and insulin-like growth factor 1 (IGF-1) concentrations were diminished. The liver synthesized IGF-1 after stimulation by growth hormone (GH) from the pituitary gland. The GH binds to the hepatic growth hormone receptor (GHR) and initiates the Janus kinase-signal transductors and activators of transcription (JAK-STAT) pathway leading to IGF-1 secretion. The JAK-STAT signalling pathway is controlled via negative feedback by suppressor of cytokine signalling (SOCS) proteins. Therefore, it was hypothesized that components of the somatotropic axis were modulated by an N-reduced diet. Seventeen male goats were divided in two groups, receiving either an adequate or a reduced N supply for 8 weeks. Serum IGF-1 concentrations were analysed by RIA assay. Concentrations of plasma GH were analysed by ELISA. The mRNA expression of hepatic GHR1A, STAT5B, JAK2, SOCS1, SOCS2 and SOCS3 was determined by *q*PCR. Data were analysed by unpaired Student's t-test. Serum IGF-1 levels were decreased due to the N-reduced feeding although plasma GH levels were not affected. The mRNA expression of hepatic GHR1A was diminished in the N-reduced fed animals while expression levels of hepatic JAK2, STAT5B and SOCS1 were not modulated. Additionally, SOCS2 and SOCS3 mRNA expression levels increased due to the N-reduced feeding. The decline in IGF-1 concentrations during dietary N-reduction could be mediated by reduced levels of hepatic GHR. Increased expression levels of SOCS2 and SOCS3 might contribute to diminished levels of IGF-1. Due to the non-physiological relationship between constant levels of GH and diminished IGF-1 concentrations, a modulation and therefore a decoupling of the components of the somatotropic axis by an N-reduced feeding seem possible in young goats.

1) Muscher AS and Huber K (2010): J Steroid Biochem Mol Biol 121, 304-307
2) Elfers K, Wilkens MR, Breves G, Muscher-Banse AS (2015): Br J Nutr 114, 1949-1964

V05

Effect of short chain fatty acids and ß-hydroxybutyrate on gene expression of gluconeogenic enzymes in the bovine liver cell line BFH12

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The energy metabolism of dairy cows is an important factor in different metabolic diseases. For a sufficient energy supply short chain fatty acids (SCFA), especially acetate, propionate and butyrate, are produced in the rumen of cattle. The proportion of SCFA is influenced by the feed composition. A crude fiber rich diet leads to increased acetate formation. Propionate as the main substrate for gluconeogenesis in ruminants rises when fed a high-concentrate diet. The key enzymes of gluconeogenesis are pyruvate carboxylase, phosphoenolpyruvate carboxykinase 1 and 2 and glucose-6-phosphatase. Another important enzyme is propionyl-CoA-carboxylase which converts propionyl-CoA to methylmalonyl-CoA. Ketone bodies, mainly ß-hydroxybutyrate (BHB), are also necessary for energy supply. BHB is produced in the ruminal wall and in the liver, particularly during periods of energy deficiency.

With our investigations we address the question whether SCFA and BHB influence the gene expression of key enzymes of gluconeogenesis in the bovine liver cell line BFH12 (Gleich et al. 2016). In first cytotoxicity studies (MTT test) we found that there is no negative effect of 1000 µmol/l acetate, 250 µmol/l propionate, 20 µmol/l butyrate and 1500 µmol/l ß-hydroxybutyrate on BFH12. In a second step we evaluated primer sets for the genes of interest and 6 housekeeping genes (18S rRNA, ß-Aktin, GAPDH, HPRT, RPL13 und SDHA) for use in quantitative PCR.

In further studies we shall investigate the effect of physiological concentrations of SCFA and BHB, alone or in combination, on gene expression of gluconeogenic enzymes.

Gleich et al. (2016) Establishment and characterisation of a novel bovine SV40 large T-antigen-transduced foetal hepatocyte-derived cell line. In Vitro Cellular & Developmental Biology – Animal, Volume 52, Issue 6, pp 662–672

V06

Dorsal shaving improves milk energy output and lowers heat stress in golden hamster females (Mesocricetus auratus)

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Golden hamster females (*Mesocricetus auratus*) produce very large litters of 3-16 altricial young and transfer large quantities of milk to them during the 3-weeks lactation period. By breeding golden hamsters we observed that subcutaneous body temperatures in lactating females were 0.5°C higher than in non-reproductive controls ($F_{1,123}=13.6$, $p<0.01$). Before energy expenditure culminates around peak lactation which occurs between days 11 and 14 in golden hamsters, we shaved females dorsally and compared their energy turnovers with females having intact fur. We observed that gross energy intake was higher as was pup growth and milk energy output. Shaved females weaned 38.5% heavier litters than litters produced by unshaved golden hamsters. Finally, we observed that with nearly identical mean litter sizes, shaved mothers had lower faecal cortisol metabolites levels so had reduced stress compared to their unshaved conspecifics. We conclude that facilitating heat loss through shaving boosts milk production and reduces stress in lactating female golden hamsters. The golden hamster provides an ideal model system where to study lactation.

V07

BET protein family member BRD4 promotes transcription through super enhancer dynamics in kidney repair and progression of fibrosis

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Background: The mammalian kidney can repair after acute kidney injury (AKI) through robust proliferation of tubular epithelial cells. Maladaptive repair can, however, lead to kidney fibrosis and chronic kidney disease (CKD). There is currently limited understanding of which transcriptional regulators activate these repair programs and how transcriptional deregulation leads to CKD. Here we investigate the existence of enhancer regulatory elements occupied by BRD4 that are activated in regenerating mouse kidney.

Methods: RNA-Seq and CHIP-Seq (H3K27ac, H3K4m3, BRD4, MED1, POL2) were performed on samples from repairing kidney cortex on day 2 after ischemia reperfusion injury (IRI) to identify activated genes, transcription factors, enhancer and super-enhancers associated with kidney repair. Further we investigated the role of super-enhancer activation in kidney repair through pharmacological BET inhibition via the small chemical compound JQ1 in vitro and in three kidney injury models in vivo.

Results: We have established the enhancer and super-enhancer landscape associated with kidney injury and repair. Furthermore, we identify key transcription factors, which cooperate with BRD4 at enhancer sites, likely activating repair programs in tubular epithelial cells. Loss of BRD4 function by systemic administration of the BET inhibitor JQ1 (50 mg/kg/day) before IRI leads to impaired recovery after AKI and increased mortality between day 2 and 3 after injury. By contrast, inhibition of prolonged transcriptional responses during the repair phase after injury, through blockade of Brd4 at enhancer sites via JQ1 starting at day 2 and day 7 after injury, ameliorates interstitial fibrosis in unilateral ureter obstruction (UUO), unilateral IRI and aristolochic acid (AA) kidney injury models at day 10 and day 21, respectively.

Conclusions: These results are the first demonstration of BRD4 enhancer and super-enhancer function in the repairing kidney, providing a critical link between AKI and CKD. In addition, our data call attention to potential caveats for use of small molecule inhibitors of BET proteins that are already being tested in clinical trials in patient at risk for AKI. Our comprehensive analysis of epigenetic changes after kidney injury in vivo has the potential to identify new targets for therapeutic intervention.

V08

Context-sensitive immunomodulation by equine multipotent mesenchymal stromal cells in a novel co-culture assay

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Multipotent mesenchymal stromal cell (MSC) therapy is being developed for a wide range of clinical applications in human and veterinary medicine. Functional characterization of the MSC is crucial and gives further insight into their mechanisms of action. Here we aimed to develop a novel co-culture assay for the assessment of MSC immunomodulatory potential, based on direct co-culture of MSC and non-sorted leukocytes obtained from whole blood.

Leukocytes from one donor horse were isolated by density gradient centrifugation optimized for recovery of all leukocyte populations including granulocytes. They were either left unstimulated or stimulated by concanavalin A (con A) or phorbol myristate acetate and ionomycin (PMA/I) for 6 h. Leukocytes were then added to equine adipose-derived MSC (N=6 donors) labelled with violet proliferation dye 450, and after 1 h of incubation allowing for cell communication, secretion of cells was blocked with brefeldin A for 4 h. All co-cultures were collected and subjected to viability and antibody staining and multicolor flow cytometry. Staining was performed for intracellular cytokines, including IL-1, TNF-α, IFN-γ and IL-10, and surface antigens to discriminate between different cell types.

Con A led to mild activation of leukocytes, whereas PMA/I led to strong activation but also decreased viability, particularly in granulocytes. Among the MSC, percentages of IL-10+ cells were increased in stimulated as well as non-stimulated co-cultures compared to MSC monoculture ($p<0.05$). Effects of MSC on leukocytes considered as anti-inflammatory included that presence of MSC decreased the percentage of IFN-γ+ monocytes and granulocytes in con A-stimulated samples (9.2±3.6% with MSC vs. 41.2% without MSC). Furthermore, MSC reduced IL-1+ granulocytes in non-stimulated (3.0±2.2% with MSC vs. 6.0% without MSC) as well as con A-stimulated cultures (2.9±1.6% with MSC vs. 13.1% without MSC). Effects with pro-inflammatory character included that a higher percentage of IL-1+ monocytes was found in non-stimulated (27.4±5.1% with MSC vs. 4.8% without MSC) as well as con A-stimulated samples (34.2±7.6% with MSC vs. 18.0% without MSC). Furthermore, IL-10+ T cells, granulocytes and monocytes were reduced by presence of MSC in non-stimulated as well as stimulated cultures.

The results demonstrate anti- as well as pro-inflammatory effects of MSC, and that there is complex context-sensitive interaction with different leukocyte subtypes.

V09

Age dependent hypothalamic and pituitary responses to novel environment stress or lipopolysaccharide in rats

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Previously, we have shown that the transcription factor nuclear factor interleukin (NF-IL)6 can be used as an activation marker for inflammatory lipopolysaccharide (LPS)-induced and psychological novel environment stress(NES) in the rat brain. Here, we aimed to investigate age dependent changes of hypothalamic and pituitary responses to NES (cage switch) or LPS (100µg/kg) in two and 24 months old rats. Animals were sacrificed at specific time points, blood and brains withdrawn and analyzed using immunohistochemistry, RT-PCR and bioassays. In the old rats, telemetric recording revealed that NES-induced hyperthermia was enhanced and prolonged compared to the young group. Plasma IL-6 levels remained unchanged and hypothalamic IL-6 mRNA expression was increased in the old rats. Interestingly, this response was accompanied by a significant upregulation of corticotropin-releasing hormone mRNA expression only in young rats after NES and overall higher plasma corticosterone levels in all aged animals. Immunohistochemical analysis revealed a significant upregulation of NF-IL6-positive cells in the pituitary after NES or LPS-injection. In another important brain structure implicated in immune-to-brain communication, namely, in the median eminence (ME), NF-IL6-immunoreactivity was increased in aged animals, while the young group showed just minor activation after LPS-stimulation. Interestingly, we found a higher amount of NF-IL6-CD68-positive cells in the posterior pituitary of old rats compared to the young counterparts. Moreover, aging affected the regulation of cytokine interaction in the anterior pituitary lobe. LPS-treatment significantly upregulated the secretion of the cytokines IL-6 and TNFα into supernatants of primary cell cultures of the anterior pituitary. Furthermore, in the young rats, incubation with IL-6 and IL-10 antibodies before LPS-stimulation led to a robust decrease of IL-6 production and an increase of TNFα production by the pituitary cells. In the old rats, this specific cytokine interaction could not be detected. Overall, the present results revealed strong differences in the activation patterns and pathways between old and young rats after both stressors. The prolonged hyperthermic and inflammatory response seen in aged animals seems to be linked to dysregulated pituitary cytokine interactions and brain cell activation (NF-IL6) in the hypothalamus-pituitary-adrenal axis.

V10
Different immune capacities of cows after polyclonal immune stimulation

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Bovine neonatal Pancytopenia (BNP) is a disease of newborn calves with an extremely high letality rate. Affected calves suffered from hemorrhagic diathesis, thrombocytopenia, leukocytopenia and bone marrow depletion. Vaccination with PregSure BVD was proven to be a cause for BNP, where upon 5-10% of the vaccinated cattle produced pathogenic BNP antibodies, which they transferred to their calves via colostrum.

In order to proof corresponding immunological differences between control cows and BVP transmitting cows, lymphocytes of PregSure BVD vaccinated controls and of BNP cows were subjected to *in vitro* polyclonal stimulation with the T-cell mitogen Concanavalin A (ConA). Lymphocytes of BNP cows showed a significantly hyper-proliferative immune phenotype after polyclonal stimulation in comparison to the vaccinated control cows. To characterize this difference in reaction on protein level, a discovery proteomics experiment with stimulated lymphocytes showed that the control lymphocytes and BNP lymphocytes differentially expressed certain proteins after ConA-stimulation.

To identify which T-cell response both cow-phenotypes develop after immune stimulation, important transcription factors and different paths of the T-cell response control lymphcytes and BNP lympho after polyclonal stimulation with ConA. We detected an increased pSTAT1 (Tyr701) expression after ConA-stimulation of the control lymphocytes as well as an elevated pSTAT3 (Tyr703) expression after the ConA-stimulation of the BNP lymphocytes.

In order to confirm the hypothesis of deviant immune phenotype being present even before PregSure BVD vaccination, PBL of non-PregSure BVD vaccinated animals were examined and we observed that 20% of unvaccinated cows showed a hyper-proliferative reaction after ConA-stimulation.

Our findings show that the deviant immunophenotype is also present in the current cow population. We should consider the fact that these cows cannot entirely eliminate pathogens after infections due to their immune response phenotype. This may result in persistent infections, for example of *Mycobacterium avium subsp. paratuberculosis* in cattle population.

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V11
Equine recurrent uveitis - Protein expression differences in effector cells

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The surface proteome of immune cells is highly dynamic and quickly adapts to changes in cell activity or differentiation. While total abundance of proteins in a cell may not considerably differ in course of cell activation, their surface abundance might show distinct alterations. Especially in autoimmune-mediated diseases, a changed effector cell surface proteome can give substantial information on possible reasons for deviant behavior of cells.

Our studies focus on effector cells in equine recurrent uveitis (ERU), an autoimmune disease in horses, which causes painful inflammation of inner eye structures and eventually leads to vision loss in affected animals due to destruction of the retina. Key players in ERU are CD4+ lymphocytes. Interestingly, as disease progresses in relapsing remitting bouts, these cells are able to migrate into the eye, despite ocular immune privilege.

To expand our knowledge on the ability of immEqune cells to overcome the blood-retinal-barrier, we compared lymphocytes from the peripheral blood stream of ERU horses with those accumulated in the vitreous. We used cell surface biotinylation to obtain the surface fraction and detected 17 proteins with higher abundance in intraocular cells, which are now subjected to functional characterization regarding ERU.

With these immune cell analyses, we aspire to contribute to a better understanding of ERU pathophysiology, supporting the development of methods for causal therapy of ERU. Furthermore, ERU shows valuable translational quality as the only spontaneous model for relapsing autoimmune uveitis in man.

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V12
Protein-protein-interaction of septin 7 and DOCK8 unravels pathomechanisms in ERU

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Equine recurrent uveitis (ERU) is a commonly occurring autoimmmune disease, which is associated with remitting painful attacks of the inner eye, eventually leading to blindness. This disease is characterized by invasion of autoaggressive T cells into the inner eye, destroying retinal structures. Since autoreactive lymphocytes are the key players in the pathogenesis of ERU, we were interested in possible changes of their protein expression pattern. Interestingly, we detected a significantly changed protein level of the GTP-binding protein septin 7, downregulated in peripheral blood lymphocytes (PBL) of ERU cases.

Septin 7 is involved in various cellular processes, including cytoskeleton organization and migration, rendering this molecule especially interesting for a potential role in autoimmune disease. In lymphocytes, however, septin 7 function is poorly characterized. Since the signaling role of septin 7 is dependent on its interaction network, we revealed novel insights into septin 7 function by the identification of DOCK8 as an interaction partner in equine PBL. DOCK8 is associated with important immune functions including the organization of cell shape as well as the regulation of immune response.

Therefore, our finding of significantly decreased DOCK8 protein level in PBL of ERU cases might explain changes in immune cell functions and shows a novel contribution of DOCK8 in spontaneous autoimmune diseases. Moreover, DOCK8 interaction proteomics in both phenotypes unraveled changes in signal transduction pathways, which are associated to autoimmune reactions in ERU. Interestingly, we detected an enhanced enrichment of the integrin pathway in PBL of ERU horses and the signal transduction molecule Integrin-linked kinase (ILK) was identified in autoimmune cases exclusively. Since ILK is involved in the regulation of proinflammatory signaling, the altered enrichment and increased ILK protein level in PBL of ERU cases might result in autoreactive inflammatory processes.

In summary, our findings provide deeper insight into interaction networks in lymphocytes and contribute to a better understanding of septin 7 function and its novel interactor DOCK8 in autoimmune disease. The decreased expression levels of these potential key players in ERU and the deviant signaling pathways driven by DOCK8, might promote autoimmunity.

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V13

Comparison of neutrophil extracellular trap formation in response to Trypanosoma cruzi infection in dogs and opossums

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The first line of defense against infectious diseases in humans as well as animals is mediated by neutrophils, which are well known as multitasking cells. They can mediate antimicrobial activity by different strategies depending on the pathogen they encounter. Besides phagocytosis, a key strategy against extracellular pathogens is the formation of neutrophil extracellular traps (NETs). Those NETs mainly consist of DNA that is decorated with antimicrobial components and mediates entrapment, growth inhibition or even killing of various pathogens. In the last years various studies about NET formation in response to bacteria, viruses and parasites have been described. Nevertheless the complete understanding if and how NET formation helps the immune system to counteract against invading pathogens and especially intracellular parasites is not fully understood and sometimes questioned.

Therefore, the goal of this study was to compare NET formation in response to the intracellular parasite *Trypanosma (T.) cruzi*. As mammals are known to be infected with *T. cruzi*, we investigated the NET formation in two *T. cruzi* reservoirs namely dogs as domestic animal and common opossums as wild animal. Neutrophils were harvested from fresh blood by density gradient centrifugation and afterwards incubated with *T. cruzi*. The analysis was conducted by immunofluorescence microscopy. Here we show that *T. cruzi* efficiently induces NET formation in neutrophils derived from opossum as well as dog blood. Both host neutrophils formed significant more NETs in response to *T. cruzi* compared to negative controls.

In conclusion, the characterization of neutrophils in various animals and humans may be helpful to understand the anti-pathogenic capacity and overall role of neutrophils against zoonotic pathogens like *T. cruzi*. Since *T. cruzi* is well known to circulate over years in both analyzed animals, it may be assumed that *T. cruzi* efficiently evades NET-mediated killing. Furthermore NETs may indeed avoid spreading of the pathogen but do not play a major role in elimination of the pathogen from the blood of dogs or opossums.

V14

Effect of hypoxia on innate immune cell function: Cell type specific effects comparing neutrophils and mast cells

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The establishment of reliable primary *in vitro* immune cell culture is of special importance as results are used to clarify *in vivo* processes and, thus, can help to reduce or replace animal experiments. One key physiological factor, which is often underestimated when culturing primary cells, is the oxygen level in the inflamed or infected tissue *in vivo*. It is well known that the cellular homeostasis and the adaptation to oxygen stress are post-transcriptionally regulated by the transcription factor hypoxia-inducible factor-1α (HIF-1α), which was also shown to support the antimicrobial activity of immune cells including infiltrating neutrophils and tissue resident mast cells. Here we demonstrate that primary human blood- derived neutrophils and primary murine bone marrow-derived mast cells adapt to the early phase of oxygen stress in a HIF-1α independent manner. To understand the antimicrobial function of both immune cell types against *Staphylococcus (S.) aureus* under physiological oxygen levels, cells were temporarily (3h) incubated under hypoxia (1% O_2), mimicking the acute phase of an infection in comparison to normoxia (21% O_2). Neutrophils were stimulated with phorbol 12-myristate 13-acetate (PMA) or *S. aureus* to release neutrophil extracellular traps (NETs), released nuclear DNA with the ability to mediate bacterial entrapment. The amount of spontaneous as well as of PMA induced NETs were clearly reduced under hypoxia. Going in line with the decreased spontaneous NET formation, the cholesterol level was significantly increased under hypoxia. In contrast to the neutrophil data, neither the release of mast cell extracellular traps nor the mast cell lipid composition was altered under hypoxia. However, hypoxia increased the secretion of the pre-stored mediator histamine but reduced the release of TNF-α in mast cells. For both cell types, HIF-1α level remained unchanged.

Remarkably, our results demonstrate that the setup of antimicrobial activities and lipid alterations are affected in a cell type specific manner, whereas the HIF-1α levels remain unchanged. These data highlight the importance of hypoxic oxygen levels during *in vitro* infections.

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V15

Primary culture of the rat substantia gelatinosa as a tool to investigate the effects of inflammatory stimulation on the afferent somatosensory system

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Nociceptive and thermoreceptive information is transmitted by afferent axons of dorsal root ganglia to neurons in the dorsal part of the spinal cord (*laminae* I and II termed *substantia gelatinosa*). One maladaptive consequence of inflammatory stimulation of the afferent somatosensory system is the manifestation of neuropathic pain. We, therefore, established and characterized a neuro-glial primary culture of the rat *substantia gelatinosa* and tested the responses of this structure to inflammatory stimulation with lipopolysaccharide (LPS). Primary cultures were employed for measurements of intracellular calcium. We further measured LPS-induced release of tumour necrosis factor-α (TNF) and interleukin-6 (IL-6) into the supernatants and investigated the activation of the inflammatory transcription factors NF-κB, STAT3 and NF-IL6 by means of immunocytochemistry.

About 80% of all investigated neurons from the *substantia gelatinosa* responded to glutamate with a transient increase of intracellular calcium, while substance P evoked a calcium response in a smaller population of neurons (43%). Both of these transmitters are responsible for the transfer of peripheral nociceptive signals to the neurons in the spinal cord. We also identified putative neurons of the thermoafferent system, which responded with calcium signals to external warming from 37-45°C (18%) or cooling from 37-25°C (6%). Neurons of the primary culture from the *substantia gelatinosa*, thus, showed the proposed responses to nociceptive and thermal stimulation. Incubation of the cultures with LPS for 2 or 4 hours resulted in pronounced release of TNF-α and IL-6 into the supernatant. Immunoreactive TNF could be visualized in the perinuclear Golgi complex of microglial cells from LPS-stimulated primary cultures. Upon stimulation with LPS, we further observed nuclear translocations of NF-κB and NF-IL6 in microglial cells and of STAT3 in astrocytes of the cultured *substantia gelatinosa* cells, indicative of an activation of these transcription factors under inflammatory conditions.

Having established a primary culture of the rat *substantia gelatinosa* and having characterized the responses to inflammatory stimulation, we are able to test modulatory influences of stimulus-induced cellular responses to various mediators or drugs with suggested pro- or anti-inflammatory capacities.

V16

Existence of a local renin-angiotensin system in the hypothalamic Organum subfornicale: functional characterization of a SFO-specific primary cell culture system

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The *Organum subfornicale* (SFO) plays an eminent role in the central control of body fluid and cardiovascular homeostasis through sensing circulating angiotensin II (AngII) as active component of the renin-angiotensin system (RAS). Activated SFO neurons relay perceived information to hypothalamic structures with final induction of thirst, peripheral vasoconstriction and renal Na^+ retention.

To additionally characterize the potential expression of all major components of a central RAS [renin, angiotensinogen (aogen), converting enzyme (ACE1), other enzymes, AngI, AngII], neurons and astrocytes of a primary rat SFO cell culture were subjected to continuous microspectroscopic analysis of induced intracellular calcium signaling (elevated $[Ca^{2+}]i$) using the fura-2-AM ratio imaging technique.

To elucidate functional expression of RAS components, SFO cells were stimulated by short-term superfusion with the appropriate substrate [aogen, Ang(1-12), AngI] for each enzyme [renin, ACE1, chymase/cathepsins] in the absence and presence of specific enzyme inhibitors (aliskiren, chymostatin, captopril). Final AngII superfusion was used as control. The following results were achieved:

- Activation with aogen (10^{-6} M) was completely / partially inhibited by aliskiren in neurons (53 %) and astrocytes (46 %), indicative of renin expression.
- Activation with aogen (10^{-6} M) was completely / partially inhibited by chymostatin in neurons (48 %) and astrocytes (50 %), indicative of chymase / cathepsin expression.
- Activation with AngI (10^{-6} M) was completely inhibited by captopril in all neurons and astrocytes analysed, indicative of ACE1 expression.
- Activation with Ang(1-12) (10^{-6} M) was completely / partially inhibited by chymo-statin in neurons (46 %) and astrocytes (40 %), indicative of chymase / cathepsin expression.
- Activation with Ang(1-12) (10^{-6} M) was completely / partially inhibited by captopril in neurons (48 %) and astrocytes (61 %), indicative of ACE1 expression.

The results obtained support functional expression of a SFO-intrinsic RAS.

Immunocytochemical characterization of primary SFO cultures shows that AngII is mainly present in GFAP positive cells (astrocytes) and aogen can be detected in vimentin positive but GFAP negative cells (tanycytes, ependymocytes).

V17
Investigation of the NH4+ conductance of the human analogue of TRPV3

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Introduction: The Olmsted syndrome is a rare hereditary skin disease observed in patients with a gain of function mutation of the hTRPV3 channel, leading to severe hyperkeratosis. However, the details of the pathogenesis of this disease and others linked to mutations of hTRPV3 remain obscure. In particular, it is unclear if hTRPV3 plays a role in conducting NH_4^+ as a major product of protein metabolism.

Methods: *Xenopus* oocytes were injected either with a linearized strep-tagged hTRPV3-cRNA transcript or with RNase-free water as control. The expression was verified using western blotting. In *Xenopus* oocytes, membrane potential and pH_i was measured using double-barreled pH-sensitive microelectrodes. Additionally, membrane patches were investigated to determine the single channel conductance for NH_4^+.

Results: In NaCl solution, the pH_i of hTRPV3 overexpressing oocytes (7.57 ± 0.03) was similar ($p = 0.16$) to controls (7.62 ± 0.03). All oocytes showed a significant depolarization ($p \leq 0.001$) and acidification ($p \leq 0.001$) when treated with NH_4Cl solution for ten minutes, after which oocytes expressing hTRPV3 had a significantly ($p = 0.013$) lower pH_i (6.00 ± 0.11, n/N = 16/3) than controls (6.36 ± 0.09, n/N = 17/3). Furthermore, the hTRPV3 oocytes had a significantly ($p = 0.010$) higher relative permeability of Na^+ versus $NMDG^+$ (1.94 ± 0.17) than the controls (1.39 ± 0.12).

Single channel conductances of patches from oocytes were significantly higher after expression of hTRPV3 ($p = 0.036$ for Na^+ and $p = 0.016$ for NH_4^+). In 13 hTRPV3 patches, large conductances of 91 ± 8 pS for Na^+ and 190 ± 15 pS for NH_4^+ were observed. Eleven patches showed conductances lower than 100 pS (25 ± 4 pS for Na^+ and 38 ± 6 pS for NH_4^+) indicating endogenous channel activity. Five of these patches also showed large channel events. Eleven patches showed no single channel activity. Conversely, 12 out of 21 control patches showed endogenous channels; no patch had a conductance over 95 pS.

Conclusion: Channel-mediated uptake of NH_4^+ has be considered as a major pathway for the exchange of ammonia in cellular systems, with the conductance of hTRPV3 for NH_4^+ of 190 ± 15 pS. Given that TRPV3 is highly expressed by the keratinocytes of the human skin, implications may follow for the pathophysiology of certain skin diseases.

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V18
Butyrate - regulator of pathways in ovine ruminal epithelium?

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Short chain fatty acids (SCFA) are long known as the main energy source of ruminants. Nonetheless, recent findings in human and rodent colon research indicate not only a huge nutritional role but also an influence of SCFAs on mediating intracellular pathways. One potential mechanism could be by activation of free fatty acid receptors (FFAR) that lead to lowered levels of cyclic adenosine monophosphate (cAMP) through inhibition of adenylyl cyclases.

Stripped ruminal epithelia obtained from ventral sac of sheep were mounted in Ussing chambers. In the first set of experiments, tissues were incubated with forskolin (an adenylyl cyclase stimulator) followed by either 1mM niacin or 10mM butyrate on both sides of epithelium or 10mM butyrate either serosally or mucosally. In the second set, influence of only mucosal application of niacin or butyrate under mucosal pH values of 6.5 or 7.4 was determined. After incubation, epithelia were separated from subepithelial layers and minced in lysis buffer. Determination of cAMP levels in the supernatant was performed using AlphaScreen cAMP Assay Kit (PerkinElmer). Degradation of cAMP was inhibited by adding 3-isobutyl-1-methylxanthine, an inhibitor of phosphodiesterase, to all buffers.

In the following, treatment data are compared to only forskolin stimulated epithelia. High cAMP levels, provoked by 10µM forskolin, were significantly lowered by subsequent addition of butyrate on mucosal side by 42%. Addition of butyrate or niacin on both sides led to a not significant yet notable drop of 23% or 29%, respectively. To elucidate whether pH might effect cAMP, we incubated tissues with forskolin and butyrate or niacin only mucosally, with mucosal buffer solution of pH 7.4 or 6.5. Whereas addition of niacin showed no effect at either pH, combination of low mucosal pH and 10mM butyrate led to a significant drop of 54% compared to only forskolin stimulation at low pH.

The strong inhibitory influence on cAMP level of mucosally added butyrate in combination with low mucosal pH reinforce the hypothesis of butyrate acting as a mediator of intracellular pathways. Lowered cAMP levels after application of niacin on both sides, but not after only mucosal addition lead to the assumption that FFAR might only be partly responsible for this. Which other mechanisms are potentially involved and which functional consequences emerge from this still need to be elucidated.

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V19

Effects of the absorption enhancer caprate on follicle-associated epithelium of porcine Peyer's patches

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Outline: Peyer's patches (PP) are part of the gut associated lymphoid tissue. They are located within the mucosa of the distal small intestine and control the interaction between antigens and the immune system. The follicle-associated epithelium that covers PP is characterized by a rather strong barrier function, when compared with surrounding villous epithelium (VE)[1]. Absorption enhancers such as the medium-chain fatty acid caprate can be employed to permeabilize intestinal barriers to enable uptake of therapeutic substances[2]. This study aimed at a comparative approach regarding the influence of caprate on epithelial barrier function in PP and VE to determine potential pro-allergenic effects of the compound.

Methods: Samples of porcine PP and VE taken from adult individuals were mounted in Ussing chambers. In parallel approaches, either 0.5, 1 or 5 mM caprate was added to the apical side. Measurements of transepithelial resistance and paracellular flux of sodium fluorescein were carried out during a 4h incubation period, respectively. Protein samples were analyzed by immunoblotting using specific antibodies raised against tight junction proteins.

Results: After addition of caprate to the mucosal compartment no long term effects of 0.5 and 1 mM caprate on transepithelial resistance and paracellular flux of sodium fluorescein could be detected. Remarkably, 5 mM caprate lead to a significant increase of TER in PP samples, though. In all cases paracellular permeability of the flux marker remained stable. The expression of the tightening tight junction proteins claudin-3, -5 and tricellulin was not significantly changed.

Conclusion: Although caprate has been shown to reversibly decrease the transepithelial resistance and increase the paracellular permeability in intestinal cell monolayers[2], results of measurements in porcine intestine were rather inconspicuous for the lower concentrations. However, caprate appears not to enhance the allergenic susceptibility in intestinal tissue. Another approach focusing on molecular regulation may enlighten further aspects of caprate effects on cell- and segment-specific intestinal epithelial barrier regulation in intestine.

1) Radloff J, Falchuk EL, Markov AG, Amasheh S (2017). Front Physiol. 8:579

2) Krug SM, Amasheh M, Dittmann I, Christoffel I, Fromm M, Amasheh S (2013). Biomaterials 34:275-282

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V20
Hypoxia in jejunum epithelium: fast adaptation of glucose transport

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The gastrointestinal epithelium is known to tolerate huge variations in oxygen supply, therefore it must command elaborate adaptation mechanisms. We hypothesized that under hypoxia import of glucose as the main fuel into the intestinal epithelial cells must be tightly regulated by these mechanisms.

We incubated isolated lagomorph jejunum epithelia in Ussing chambers under short-circuit-conditions. After equilibration, we simulated hypoxia by gassing a group of epithelia with 1% O_2 + 99% N_2. We assessed electrogenic transport of glucose via sodium-coupled glucose transporter (SGLT) 1 by measuring the increase of short-circuit current (I_{sc}) after mucosal addition of glucose as well as its transepithelial transport using ^{14}C-glucose. Additionally, we incubated the epithelia with inhibitors for different glucose transport mechanisms and evaluated their effect on ^{14}C-glucose transport.

We found a significant decrease in the glucose-induced changes of I_{sc}, i.e. a decreased activity of SGLT 1 under hypoxia compared to the control group. Despite the reduced activity of SGLT1, the transepithelial transport of ^{14}C-glucose was not diminished by hypoxic conditions, indicating that SGLT 1 was supplemented by other non-electrogenic transport mechanisms. An inhibition of transepithelial glucose transport by a specific inhibitor of glucose transporter (GLUT) 1 under hypoxic but not under control conditions revealed an involvement of GLUT 1. Because these changes were observed after only 45 minutes of hypoxia, the adaptation must be mediated on the protein level. Thus, we assessed the influence of AMP-activated protein kinase (AMPK) by preincubating the epithelia with its antagonist compound C before submitting them to hypoxia. By doing so, we could abolish the decrease in SGLT 1 activity under hypoxia. Western blot studies revealed a phosphorylation, i.e. activation of AMPK under hypoxia that was also abolished by compound C.

Summing up, we could show that glucose transport mechanisms in jejunum epithelium are modulated quickly under hypoxic conditions. A substitution of the energy-dependent import via SGLT 1 by GLUT 1 mediating facilitated diffusion might help to secure an energy-saving uptake of the cells' most important fuel and thus maintaining their function. This adaptation process seems to be mediated by AMPK.

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V21

Effects of feeding graded levels of deoxynivalenol and oral administration of lipopolysaccharide on expression of barrier function and innate immune genes in the small intestine of chickens

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The mycotoxin deoxynivalenol (DON) is frequently found in animal feeds. Ingestion of DON impairs gut health and functioning in poultry, including intestinal transport mechanisms, barrier function and the immune response. Lipopolysaccharides (LPS) are part of the outer membrane layer of Gram-negative bacteria with modulatory properties of the innate immune system. The aim of this study was to investigate the effects of feeding 4 different low to moderate DON-levels and an oral LPS challenge on the mucosal expression of genes for tight junction proteins and innate immunity in the duodenum and jejunum of broiler chickens. In total, 80 day-old Ross 308-broilers were allocated to 4 feeding groups in a 4×2 factorial design, receiving 0 ppm, 2.5 ppm, 5 ppm and 10 ppm DON in the diet until week 5 of life. Twenty-four hours before sampling, half of the birds received an oral LPS challenge (1 mg LPS from *E. coli* O55:B5/kg body weight), whereas the other half received a placebo treatment. Intestinal mucosa samples were collected from the duodenum and jejunum of 5-week-old chickens and the relative expression of 12 target genes was measured. The expression of claudin 1 was upregulated in both duodenum and jejunum of DON-fed animals ($p < 0.05$). Expression of Toll-like receptor 2 was increased in the duodenum of chickens fed the DON diets compared to chickens receiving the control diet, whereas it was linearly down-regulated with increasing DON concentrations in the jejunum ($p \leq 0.05$). Transforming growth factor-β1 expression was quadratically affected by the increasing DON concentrations in the jejunum ($p < 0.05$), with the lowest expression with the 2.5 and 5 ppm diets. The LPS challenge upregulated the duodenal expression of occludin by 9.7 % ($p < 0.05$). In conclusion, the exposure of the duodenum and jejunum to DON and LPS modulated the mucosal barrier. As innate immune genes were differently expressed at the two gut sites, this may have been associated with DON-related effects on the gut microbiota and microbial DON metabolism.

V22
Permeation of butyrate across the ovine reticulum epithelium

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In the forestomach of ruminants, carbohydrates are broken down by microbial fermentation. The main products of these processes are short-chain fatty acids (SCFA = acetate, propionate, butyrate) with butyrate showing a portion of 10 – 15 % among the three SCFA when regarding the concentration. SCFA form the basis for ruminant´s energy maintenance and are absorbed for the most part directly from the reticulorumen (rumen + reticulum). While the mechanisms of SCFA-absorption across the ruminal wall were in focus of research in the last decades, less is known about the SCFA-absorption in the second part of the forestomach, the reticulum.

For our experiments, we used stripped ovine reticulum epithelia obtained from female merino breed sheep of different age. Epithelia were mounted in Ussing-chambers under short-circuit conditions. Fluxes of radiolabelled butyrate across the reticulum epithelium were measured in mucosal to serosal (Jms) and serosal to mucosal direction (Jsm). To inhibit Na-H-exchanger, monocarboxylate transporters (MCTs), sodium-coupled monocarboxylate-transporters (SMCTs) or the Na-K-ATPase, we applied EIPA, pHMB, fenoprofen or ouabain, respectively. Electrophysiological parameters were monitored throughout the experiment.

We observed a net absorption of butyrate (Jms = 1.87 ± 0.35 µmol cm^{-2} h^{-1}; Jsm = 1.12 ± 0.12 µmol cm^{-2} h^{-1}, p = 0.013). Incubation with ouabain abolished short-circuit current and reduced Jms of butyrate by about 32 % (0.69 ± 0.19 µmol cm^{-2} h^{-1}, p < 0.001). Jsm of butyrate was not affected by ouabain treatment. pHMB reduced Jms of butyrate from 2.02 ± 0.4 to 1.29 ± 0.22 µmol cm^{-2} h^{-1} p = 0.005) but did not affect Jsm of butyrate. Both Jms and Jsm of butyrate were reduced in tendency by fenoprofen application (about 10 % reduction, p = 0.05). EIPA did not affect Jms of butyrate but reduced Jsm of butyrate by about 11 % (p = 0.018).

Our results show that butyrate is efficiently taken up by the reticulum epithelium. The mechanisms behind are at least partially sodium and/or voltage dependent. The results of our inhibitor studies show that MCTs and SMCTs could be involved in the permeation of butyrate across the reticulum epithelium. Furthermore, our results suggest that the reticulum epithelium is functionally different from the rumen epithelium.

V23

Dietary methionine source influences the expression profile of methionine transport systems in the gastrointestinal tract of growing pigs

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Introduction: Methionine (Met) is an essential amino acid (AA) that often requires dietary supplementation in farm animals for optimal health and performance. A previous study showed that dietary DL-Met supplementation increased Met absorption in the porcine small intestine. The present project explored the distribution of various Met transporters along the gastrointestinal tract (GIT) of growing pigs supplemented with either L-Met, DL-Met or the Met precursor DL-2-hydroxy-4-(methylthio)butanoate (DL-HMTBA).

Methods: A total of 27 piglets (10 weeks old) were randomly allocated to 3 feeding groups receiving diets supplemented with either 0.21% DL-Met (diet 1), 0.21% L-Met (diet 2) or 0.31% DL-HMTBA (diet 3) to meet Met+Cys requirement. Expression analysis of selected Met transport proteins was conducted in 11 gastrointestinal (GI) regions using quantitative real-time PCR and, in part, by Western blot.

Results: Relative gene expression was different among the GI sections for all genes evaluated ($P < 0.001$); however, ASCT2, SNAT2 and IMINO showed only moderately heterogeneous expression across GI segments. The B^0AT1, $b^{0,+}$, y^+LAT1 and LAT4 showed high expression in the small intestine with B^0AT1, y^+LAT1, LAT2 and LAT4 dominating in the mid-jejunum, and $b^{0,+}$ dominating in the proximal jejunum. In the small intestinal segments, the mRNA expression of ASCT2 was increased by diet 1 and the mRNA expression of LAT2 was increased by diet 3. The protein levels of B^0AT1 in the small intestine were greatest in the ileum ($P < 0.001$) wherein they were increased by diet 1 ($P < 0.001$). LAT4 protein was increased in small intestinal and extraintestinal regions (oral mucosa, stomach) by diet 3.

Conclusion: A diet containing DL-Met upregulates the Na^+-dependent apical systems ASCT2 at the mRNA level and B^0AT1 at the protein level in the small intestine. A diet containing DL-HMTBA is linked to higher expression of the basolateral systems LAT4 and LAT2 in the small intestine which can help to provide enough Met to the epithelial cells via blood because these cells do not receive free Met from diet. The higher expression of ASCT2 and/or B^0AT1 could provide an explanation for the higher Na^+-dependent Met transport in pigs fed a DL-Met-containing
diet.

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V24
Impact of inflammation on neuro-immune interactions in the intestine of rats

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Recently, the impact of mast cells on gastrointestinal disorders, e.g. food allergy or inflammatory bowel diseases (IBD), has come in the focus of research. Intestinal mast cells contain different pro-inflammatory mediators, which are either stored in granules or will be synthetized de novo. After stimulation, these mediators are released and they interact with different cells, e.g. submucosal neurons. This leads to an increased secretory influx of ions and water into the gut lumen (secretory diarrhoea), which is one of the main symptoms of IBD.

One of the first steps in the pathogenesis of IBD is an impaired epithelial barrier, which leads to a higher density of mast cells in the intestinal wall. The role of these mast cells during IBD is still unclear and will be investigated in the present study.

An animal model, consisting of two groups of 6-8 week old rats, was used. In the first group the rats were sensitized solely against ovalbumin, while in the second group a mild colitis was additionally induced by rectal administration of TNBS (2,4,6-trinitrobenzenesulfonic acid). Mucosa-submucosa preparations of distal colon and jejunum were taken. Their anion secretion and conductance were measured in Ussing chamber experiments. Further experiments were carried out to quantify the mucosal and submucosal mast cells by performing immunofluorescent staining with c-kit antibody and qPCR. Antibodies against claudin-1, -3, -4, -8 were used to investigate the epithelial barrier function.

In comparison to the solely sensitized group, the colitis group showed a reduced response to ovalbumin in Ussing chamber experiments and reduced number of c-kit positive mast cells. The basal conductance, which is a marker for the epithelial permeability, had significantly increased during colitis. Hence, the epithelial barrier function was investigated by measuring the intensity of claudin-1, -3, -4 and -8, which act as sealing proteins in the intestinal epithelium. The quantification of claudin-3 and -4 showed an upregulation in both experimental groups compared to control, but in the colitis-group there was a reduced claudin-3 intensity compared to sensitization.

From our results we concluded that different mediators or receptors might be involved in the interactions between intestinal mast cells and prosecretory neurons. Hence, further experiments are being conducted to understand the function of mast cell mediators and receptors involved in this interaction.

V25

Influence of dietary menthol-based bioactive lipid compounds on conductance and uptakes of glucose and methionine in the rumen and intestine of sheep

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The present study was conducted to investigate if dietary plant bioactive lipid compounds (PBLC) with menthol as a lead substance could influence the uptakes of glucose and methionine (Met) and electrophysiological properties of the rumen and intestine of sheep.

Methods: Growing Suffolk sheep (n = 24) were equally distributed to three diet supplemented with no PBLC (diet 1), 80 mg/d of PBLC (OAX17, PerformaNat GmbH; diet 2) and 160 mg/d of PBLC (diet 3) in a randomised block design based on initial body weight and sex. After 28 d of supplementation, epithelia from the ventral rumen and mid-jejunum were collected for ex vivo measurements of conductance (G_t) and glucose and Met uptake using Ussing chambers. Uptakes of glucose (200 µM) and Met (50 µM) were measured in the presence or absence of Na on the mucosal side. Data were analysed using mixed model procedures of SAS.

Results: In the rumen, baseline G_t increased linearly with increasing doses of PBLC (3.21, 3.62 and 4.37 mS/cm^2 for diet 1, 2 and 3, respectively; linear P = 0.023 and SE = 0.34). Glucose uptakes in the presence (mean = 254 pmol/cm^2/min; linear P = 0.55; SE = 43.3) or absence (mean = 217 pmol/cm^2/min; linear P = 0.11; SE = 21.4) of Na^+ were not affected by the PBLC. Met uptakes were also not affected by the PBLC in the presence of Na^+ (mean = 62.7 pmol/cm^2/min; linear P = 0.40; SE = 12.2), but showed a linear increase due to increasing doses of PBLC (50.3, 45.7 and 67.9 pmol/cm^2/min for diet 1, 2 and 3, respectively; linear P = 0.05; SE = 5.92) in the absence of Na^+. The absence of Na^+ tended to decrease ruminal glucose (P = 0.10) but not Met uptake (P = 0.24).

In the jejunum, Gt and uptakes of glucose and Met in the presence or absence of Na^+ were not influenced (P > 0.10) by the PBLC. Intestinal uptakes of glucose (1283 vs. 827 pmol/cm^2/min; SE = 82.4; P < 0.001) and Met (727 versus 421 pmol/cm^2/min; SE = 46.2; P < 0.001) were different in the presence vs. absence of Na^+.

Conclusions: Supplementing PBLC exert an enhancing effect on the ion permeability of the ruminal epithelium and no effect on glucose uptake. The Na^+-independent absorption of Met in the rumen appears to be increased by PBLC; however, ruminal Met uptake is very small and not clearly Na^+-dependent. No effects of PBLC were evident on the investigated variables in the intestine.

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V26

Campylobacter jejuni modulates the intestinal mucosal barrier function with consequences on bacterial translocation in chickens

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The intestinal epithelial barrier serves as the first line of defence between the host and the luminal environment. There is considerable evidence that a dysfunction of the intestinal barrier is an important factor contributing to the pathogenicity of some enteric bacteria (e.g. Campylobacter jejuni). For a long time Campylobacter was only considered as a commensal in avian hosts restricted to the ceca, without any pathogenic features. However, recent studies challenge this belief. In experimental studies it was noticed for some *Campylobacter* isolates that the colonization of the gut has a negative impact on birds' health and well-being. For example, *C. jejuni* disrupts the intestinal barrier which results in a higher intestinal permeability. This effect promotes the translocation of bacteria to internal organs, an effect not solely restricted to Campylobacter itself. Accordingly, not only C. jejuni can cross the intestinal epithelial barrier, but the translocation of other enteric microorganisms such as E. coli to extra-intestinal organs is facilitated as well. Changes in the intestinal barrier function characterized by increased intestinal permeability, downregulation of certain nutrient transporters and an induction of mucous production display severe consequences on nutrient uptake. Consistent with the latter facts, it was found that glucose uptake and amino acid availability were reduced following C. jejuni infection. Furthermore, it was revealed by two-photon microscopy that intracellular calcium $[Ca^{2+}]_i$ was highly up-regulated in the intestinal mucosa of infected birds, indicating that the modulation of $[Ca^{2+}]_i$ by Campylobacter might be involved in facilitating the necessary cytoskeletal rearrangements that occur during the bacterial invasion of epithelial cells. Moreover, C. jejuni colonization could be associated with an alteration of the gut microbiota as infected birds had a significantly lower abundance of E. coli at different gut sites. On the contrary, the level of Clostridium spp. was higher in infected birds compared with control birds, demonstrating that the infection of chickens with C. jejuni was associated with significant changes in the composition of the intestinal ecosystem. Altogether, these findings indicate a somewhat intense interaction between C. jejuni and its avian host. In fact, the Campylobacter-host interaction in the gut is characterized by a loss of integrity of the mucosal barrier.

V27

Effects of lactic acid treatment of cereals and dietary phytase on phosphorus digestibility and bone parameters in growing pigs

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Although comprising a substantial amount of phosphorus (P), most of the P in cereal grains is in the form of phytate-P which has a low digestibility in monogastric animals. To reduce the addition of inorganic P in diets for pigs due to environmental concerns, the most common dietary strategy to increase the intestinal availability of phytate-P is to supplement pig diets with microbial phytase. Another strategy may be soaking of cereal grains in mild organic acids, such as lactic acid (LA), which are already used to enhance gut health in weaned pigs and as feed preservative. Therefore, the present aim was to investigate whether soaking of cereal grains in LA may enhance the intestinal P digestibility and bone characteristics in pigs and whether this effect was comparable to dietary phytase supplementation. Thirty-two growing barrows were assigned to 1 of 4 diets (2 pigs per diet/ run) in a 2 ´ 2 factorial design: 1) diet with untreated wheat and corn and without phytase (Con diet); 2) Con diet with phytase (Con-Phy diet); 3) diet with LA-treated wheat and corn and without phytase (LA diet); 4) LA diet with phytase (LA-Phy diet). The conditions for LA treatment were soaking of the whole grains in 2.5% LA for 48 hours and drying for 24h. Pigs were meal-fed 3-times daily and had ad libitum access to demineralized water. The LA treatment of cereals tended ($p = 0.078$) to improve pig's FCR by 6.7%. Feeding phytase increased the apparent total tract digestibility (ATTD) of crude ash, P and calcium by 14.4%, 49.3% and 17.7% compared to the diets without phytase, respectively ($p < 0.001$). Moreover, pigs fed the LA diet had a 0.9% greater ATTD of P compared to pigs fed the Con diet, whereas pigs fed the LA-Phy diet even had a 5.3%-higher ATTD of P compared to pigs fed the Con-Phy diet ($p < 0.05$). However, dietary treatments did not affect the length and weight of femurs and metacarpals. In conclusion, albeit the phytase supplementation was more efficient, LA treatment of cereals has the potential to improve the digestibility of P in growing pigs and further potentiate the effect of phytase on ATTD of P.

V28

A four-week high-concentrate feeding alters hindgut microbiome leading to dysbiosis in dairy cows

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Introduction: Feeding of starch-rich diets to dairy cows increases milk yield but also overwhelm the digestive capacity of the rumen. This leads to rumen fermentation disorders and also greater substrate amounts bypassing the rumen, which challenge the normobiosis in the hindgut. Alterations of the microbiome, commonly known as dysbiosis, are increasingly seen as a risk factor of metabolic perturbations in cattle. The aim of this study was to investigate at which extent a constant high-concentrate (HC) feeding of 4 weeks affect ruminal pH and the fecal bacterial community and fermentation parameters in dairy cows.

Materials and Methods: Sixteen lactating Simmental cows (90.9±22.1 days in milk) were first fed a Baseline diet (40% concentrate, 60% forage; DM basis) for 1 wk, followed by a HC diet (60% concentrate, 40% forage) for 4 wk. Ruminal pH was recorded continuously using indwelling sensors. Fecal samples for pH and short chain fatty acids (SCFA) measurements were taken in Baseline, HC wk2, wk3, and wk4, for microbiome analysis in Baseline, HC wk2, and wk4. Fecal pH was measured using a hand-held pH meter, SCFA were analyzed using gas chromatography. Bacterial DNA was extracted and sequenced with MiSeq platform, targeting the V3-5 region of the 16S rRNA gene, and bioinformatic analysis was performed using QIIME. Statistical analysis was done using SAS.

Results: Ruminal pH and fecal pH decreased significantly with the HC diet, whereas fecal SCFA increased ($P < 0.01$). These results showed the clear impact on rumen and hindgut digestive physiology due to HC feeding. Phylogenetic distance analysis of microbiome data showed a clear clustering of Baseline samples compared to HC feeding samples. High concentrate feeding decreased ($P < 0.01$) the richness and diversity of the fecal bacterial community suggesting a distinct impact of diet on the community structure and dysbiosis index. Diet affected 20 of 34 taxonomically assigned families. The highest abundant family *Ruminococcaceae* (62% total relative abundance) decreased in HC wk2 ($P < 0.02$), *Lachnospiraceae* (6.1% rel.ab.) increased in HC wk2 and 4 ($P < 0.01$), and *Bacteroidacaea* (4.5% rel.ab.) decreased in HC wk2 and 4 ($P < 0.01$).

Conclusion: The HC feeding clearly impacted the hindgut bacterial community and their fermentation parameters. The increase of starch flow to hindgut led to depression of pH and also to hindgut dysbiosis in cows fed a 4-wk HC diet.

V29

Evaluating the biological sensitivity of non-invasive methods for measuring adrenocortical activity: an example in cattle

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Faecal cortisol/corticosterone metabolites (FCM) are frequently measured for non-invasive assessment of hypothalamic–pituitary–adrenal (HPA) axis activity. Unfortunately, utilized methods in roughly one third of all published papers lack a sound physiological validation (demonstrating that an increase in plasma glucocorticoid levels is well reflected in FCM concentrations). Such a validation is frequently performed by injecting a high dose of ACTH. The present study aimed to evaluate in cattle diurnal variations (n=10 cows) in FCM excretion and the biological sensitivity of an 11-oxoaetiocholanolone enzyme immunoassay (EIA) by a dose response experiment over a wide range of intravenously injected synthetic ACTH (Synacthen, CIBA-Geigy, Switzerland) amounts (n=2 each: 0.016; 0.031; 0.063; 0.125; 0.25; 0.5; 1.0 or 3.0 mg per animal). Frequent blood samples (via indwelling catheters) and all voided faeces were collected for 24 hours before (blood only in ten cows) and after the ACTH challenge. Plasma cortisol and faecal cortisol metabolites (11,17-dioxoandrostanes) were measured via EIA. Episodic variations (expressed as max. to min. and CV% in individual cows) of FCM were less pronounced than those of plasma cortisol (mean±SD of all cows: 2.4±0.4 vs. 15.4±9.3 and 26±8% vs. 82±24%, respectively). Even the lowest ACTH dose (0.016 mg) gave a clear signal in both sample types. Dose of ACTH was only well correlated ($r=0.819$; $p<0.0001$) with the percent increase (above baseline) of FCM, suggesting that their concentration is a better reflection of HPA axis responses than plasma cortisol values. Less expressed episodic variations in FCM levels warrant them better suited for assessing baseline or chronic HPA activity (especially when only a few samples can be collected). In addition, our group-specific 11-oxoaetiocholanolone EIA (especially designed to pick up a group of faecal cortisol metabolites) is characterized by a high biological sensitivity, which enables the detection of minor stressful events.

V30

The hepatocyte-derived cell line BFH12 as a new in vitro model for bovine biotransformation

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Introduction: Cattle are frequently exposed to a variety of xenobiotics, such as antibiotics, growth hormones and pesticides. Accumulation or carry-over of harmful compounds from feed to food represents a risk to public and animal health. However, little is known about the metabolism of such xenobiotics in cattle, which is likely due to the lack of an appropriate model. Recently, we established the hepatocyte-derived cell line BFH12 that exhibits many characteristics of primary cells including a hepatocyte-like metabolism. The aim of the present study was to demonstrate the potential of BFH12 as an in vitro model of bovine biotransformation.

Methods: Cells were cultured in Williams' Medium E containing 5 % heat-inactivated FBS and other additives. Gene expression of biotransformation enzymes/transporters was determined by reverse transcription PCR (RT-PCR) using total RNA from BFH12, fetal bovine hepatocytes and adult liver tissue. Presence of several genes was determined, including cytochromes P450 (CYP) 1A1, 1A2, 2B6, 2C19, 2E1, 3A4 as well as UDP glucuronosyltransferases 1A1 (UGT1A1) and A6, glutathione S-Transferase M1 (GSTM1), P-glycoprotein (P-gp), ATP-binding cassette sub-family G member 2 (ABCG2), multidrug resistance-associated protein 1 (MRP1), and sodium-taurocholate cotransporting polypeptide (NTCP). Efflux transporters were also examined by immunofluorescence. Ethoxyresorufin-O-deethylase (EROD) assay was performed to investigate CYP1A induction by different concentrations of benzo[a]pyrene. Bile acid production as a further indicator of conjugating activity was measured by mass spectrometry.

Results: RT-PCR analysis showed that BFH12 expresses mRNA of CYPs 1A1, 2C19, 3A4 as well as phase II enzymes UGT1A1, UGT1A6, GSTM1 and efflux transporters P-gp, NTCP, ABCG2 and MRP1. The expression pattern was similar to that of fetal hepatocytes. Efflux transporters ABCG2 and MRP1 were also confirmed by immunofluorescence, which indicates that BFH12 may be used for transport studies. Treatment with benzo[a]pyrene stimulated gene expression of CYP1A1 and 1A2 and increased activity of both enzymes. The bile acids taurocholic acid, taurochenodeoxycholic acid, taurodeoxycholic acid and taurolithocholic acid were identified in cell culture supernatants.

Conclusion: BFH12 retains important features of primary hepatocytes and may be a suitable in vitro model for functional studies on drug metabolizing enzymes and efflux transporters of bovine biotransformation.

V31

Comparative analysis of gestation in three rhinoceros species (Diceros bicornis, Ceratotherium simum, Rhinoceros unicornis)

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Monitoring faecal progesterone metabolite concentrations is routinely used for diagnosing pregnancy in all species of rhinoceroses kept in captivity. The length of pregnancy ranges from 15 to 16 months. For a precise analysis of gestation data results from almost 20 y of monitoring were comparatively analyzed between the Black Rhinoceros (*Diceros bicornis*), the White Rhinoceros (*Ceratotherium simum*), and the Indian or Greater One-horned Rhinoceros (*Rhinoceros unicornis*). Mean ± SEM values of gestation lengths for the three species were 461.5 ± 1.52 d; 503.0 ± 1.32 d, and 480.6 ± 1.23 d, respectively. Gestation length varied by ~7 weeks in all three species; this result is comparable to the gestation length variation in Arabian or Thoroughbred mares. Confirmed gestation lengths in rhinos ranged between 452 – 475 d; 490 – 510 d, and 462 – 497 d in the Black, the White and the Indian rhinoceros. Mean hormone values from N = 26, 34 and 23 pregnancies for the three species were calculated and revealed considerable differences in the onset of placental steroid production. Mean fecal pregnane metabolite concentrations continuously increased between days 75 – 125 in the Black, between days 65 – 150 in the White, and between days 100 – 175 in the Indian rhinoceros. Remarkably, a rather wide range in the onset of placental hormone production between days 55 – 150 was observed in individuals of all three rhino species. In conclusion, long term endocrine monitoring of gestation revealed a high degree of variability in gestation length and endocrinology of the three rhinoceros species studied.

PLENARY
POSTERS

P01

The bovine rumen expresses bTRPV3 channels as a pathway for the uptake of NH4+

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Emissions of nitrogen by dairy cattle play a considerable role in climate change. These are associated with the degradation of dietary protein into ammonium (NH_4^+) that is absorbed across the ruminal epithelium, possibly via bTRPV3 [1].

To confirm permeability of bTRPV3 to NH_4^+ [2], *Xenopus* oocytes were either injected with water (control) or strep-tagged bTRPV3-cRNA. In single channel experiments in symmetrical NH_4^+, 90% patches from bTRPV3 cells showed single channel events versus 60% of patches from controls. Large channels of 158 ± 12 pS (n = 11) could only be observed in bTRPV3 patches. When corrected for concentration, this conductance was identical to that previously obtained for NH_4^+ in HEK-293 cells expressing bTRPV3 ($p = 0.79$) [2]. Conductance to Na^+ was 101 ± 10 pS, again reflecting the concentration-corrected value in HEK-293 cells ($p = 0.84$) [2]. Additional endogenous channels were observed in both groups, but more frequently in control patches (57%; ~32 pS for Na^+; ~44 pS for NH_4^+) than in bTRPV3 patches (34%; ~36 pS for Na^+; ~48 pS for NH_4^+). Endogenous channels appeared twice as frequently in patches from *Xenopus* oocytes versus HEK-293 cells [2].

In oocytes studied with double-barreled pH-sensitive microelectrodes, the relative permeability of Na^+ or NH_4^+ versus $NMDG^+$ was significantly higher in bTRPV3 than in controls (both n = 17, $p < 0.02$). Interestingly, exposure to NH_4Cl led to a significant acidification ($p < 0.001$) in both bTRPV3 and control oocytes ($pH_i \sim 6.4$).

To investigate expression in the bovine rumen, a commercial TRPV3 antibody was used revealing staining of the stratum spinosum and granulosum. The specificity of the antibody was tested using western blotting and immunohistological staining of bTRPV3 and control *Xenopus* oocytes.

In conclusion, we suggest that bTRPV3 participates in the ruminal uptake of NH_4^+ with expression levels highest in the apical layers of the functional transporting syncytium. Furthermore, we show that the single channel conductance of bTRPV3 is similar after expression in *Xenopus* oocytes and HEK-293 cells. However, *Xenopus* oocytes, in particular, also express endogenous NH_4^+- permeable channels with a lower single channel conductance.

1. Rosendahl J., Braun H.S., Schrapers K.T., Martens H., Stumpff F.: Pflugers Arch 2016, 468:1333-1352.

2. Schrapers K.T., Sponder G., Liebe F., Liebe H., Stumpff F.: PLOS ONE (submitted 2017).

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P02

Safety and migration of intra-articularly injected mesenchymal stem cells in a focal cartilage defect model

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Purpose

Articular cartilage has a very limited capacity to self-repair. Experimental and clinical studies have shown that mesenchymal stem cells (MSCs) are able to stimulate articular cartilage repair. However, due to the lack of appropriate cell tracking models, it is still unclear whether intra-articularly injected MSC migrate and engraft in other organs. This study aimed at evaluating the *in vivo* cell migration and the safety of intra-articular delivery of MSCs into the knee joint, employing a novel immunocompetent transgenic rat model.

Materials and Methods

Bone marrow-derived MSCs were isolated from transgenic rats that ubiquitously express the genetic marker human placental alkaline phosphatase (ALPP). ALPP is a heat-stable enzyme, retaining its enzymatic activity after paraffin and plastic embedding. A focal cartilage defect was created in the immunocompetent transgenic recipient rats, which express a heat-sensitive form of the ALPP protein ($ALPP^m$). Because ALPP and $ALPP^m$ differ in only a single amino acid, transgenic rats expressing $ALPP^m$ are tolerant to cells carrying the genetic marker ALPP. $ALPP^m$ can easily be distinguished from wild-type ALPP by heat inactivation. MSCs were intra-articularly injected into the knee joints of recipient rats, and ALPP-labelled MSCs were tracked in the knees and in other organs such as lung, spleen, or heart by histochemical staining in paraffin sections after heat inactivation.

Results

ALPP-labelled MSCs were detected at the defect site, 1 week and 1 month after the cell injection, showing their capacity to migrate and engraft within the injury site. However, no ALPP-labelled MSCs could be found in the lung or in the heart at these time points, suggesting that injected MSCs do not migrate to these tissues.

Conclusion

The current study supports the safety of MSC therapy for articular cartilage repair via intra-articular delivery. Intra-articularly injected, ALPP-labelled MSCs engraft in the articular cartilage lesion, but do not migrate to and engraft in other, distant organs.

P03
Characterization of chicken TNF-a, a long missed cytokine in birds

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Members of the TNF family play crucial roles in hematopoietic cell development and immune responses to pathogens. These cytokines and their receptors have been described in vertebrates from fish to man including several avian species. However, TNFα, the founding member of the TNF family, seemed to be absent in birds. Neither functional studies nor avian genome analysis provided evidence for the existence of an avian orthologue of TNFα, thus far.

We made use of publically available genome sequences from more than 100 avian species to search for orthologues of “missing” immune genes in chickens and identified a sequence with homology to fish, reptile and mammalian TNFα. In addition, we identified orthologues of TNFR-1 and TNFR-2 in the chicken genome. Since the TNFα sequence is highly GC rich, we purchased a codon optimized expression plasmid and transfected HEK293 cells to generate the biologically active chicken cytokine. Using qRT-PCR analysis we could show that chicken TNFα is induced upon LPS treatment in the spleen and in cultured monocyte derived macrophages. Collectively, these data provide strong evidence for the existence of an avian orthologue of TNFα filling an evolutionary gap between fish and mammals.

P04
Butyrate - guardian of the porcine colon epithelium?

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Butyrate is produced in the colon epithelium by microbial fermentation. It is the key energy source for the colonocytes and has a positive influence on the homeostasis of the cells. Previous studies on rumen epithelium led to the conclusion that butyrate may also have protective effects under hypoxia, a common complication of several diseases. Hence, we hypothesized a similar effect of butyrate on the porcine colon epithelium under hypoxia. We therefore tested the influence of butyrate on tissue viability and integrity as well as gene expression of porcine colon epithelium under hypoxia *in vitro*.

Isolated porcine colonic epithelia were mounted in Ussing chambers and gassed with 100% oxygen for 15min to equilibrate. Epithelia were incubated with buffer solutions containing either 50mM Na-butyrate ('butyrate') or 50mM NaCl ('control') instead. After equilibration, chambers of each incubation group were gassed differently: 'normoxia' with 100% oxygen; 'hypoxia' with 1% oxygen and 99% nitrogen. Electrophysiological parameters (short circuit current (I_{sc}) and tissue conductance (G_t)) were measured for 2h. Subsequently, mRNA expression in the differently incubated epithelia was measured using RT-qPCR for the following genes: monocarboxylate transporter (MCT)1, MCT2, sodium-coupled monocarboxylate transporter (SMCT)1, down-regulated in adenoma (DRA), zonula occludens (ZO)1 and claudin1.

Initial I_{sc} of the epithelia treated with butyrate tended to be lower than in the control group. I_{sc} showed a significant decrease under hypoxia in both buffer solutions, but the relative decrease was significantly diminished by butyrate. Hypoxia caused an increase of G_t compared to normoxia. However, the hypoxia induced increase was significantly lower under butyrate incubation compared to the control group. Gene expression showed no significantly difference neither between different gassing nor incubation conditions.

The epithelial integrity and/or viability was reduced under oxygen depletion as illustrated by an increased G_t. In turn, a decreased I_{sc} represents a reduced electrogenic transport across the epithelium under hypoxia. The effects of hypoxia on G_t and I_{sc} were diminished by butyrate. Missing effects on gene expression levels indicate that this might be mediated by mechanisms on the protein level. Nevertheless, butyrate obviously has protective short-term effects on porcine colon epithelium under hypoxia and may prevent tissue from damage.

P05

Pre-enrichment of Mycobacterium avium subsp. paratuberculosis using plant lectins

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Mycobacterium avium subsp. paratuberculosis (MAP) is the cause of paratuberculosis or Johne's disease, a chronic inflammatory bowel disease in ruminants. Cattle with paratuberculosis show severe clinical symptoms, such as diarrhea, emaciation and lower milk production. Not only clinically ill animals but also those in a subclinical state of disease can shed high numbers of mycobacteria, leading to possible contamination of food products, particularly milk. This poses a severe threat to the consumer, as MAP cannot reliably be killed by conventional means of food safety like pasteurization. Additionally, MAP is currently discussed as a possible contributor to the pathogenesis of Crohn's disease.

The most reliable detection method of MAP in food, at this point, is by cultivation. This method can only exclude a contamination of food with MAP after eight to ten weeks, due to its extremely long growth intervals. A first step to develop a fast, sensitive and specific detection method for MAP in food, would be the pre-enrichment of MAP.

A substantial characteristic of all pathogenic mycobacteria is the presence of mannosylated lipoarabinomannans (Man-LAM) in their cellular membrane. Therefore, mannose-specific plant-lectins, like Concanavalin A (ConA) could be suitable to accumulate MAP. In order to evaluate the binding of those lectins to MAP, several plant lectins were tested in western blot and ELISA on lysed MAP, 13 control bacteria, that are frequently found in milk and other environmental mycobacteria.

ConA showed good binding to MAP, but also to other mycobacteria, like *M. avium subsp. silvaticum* and *M. avium subsp. avium*. Also other, non-mannose-specific lectins were used. We identified a total of four lectins, that showed better affinity towards MAP, than towards the control bacteria and could therefore be suited for the pre-enrichment of MAP. Two of those lectins were Datura Stramonium Lectin (DSL), which recognizes N-acetylglucosamine oligomers and Sambucus Nigra Lectin (SNA), which binds preferentially to sialic acid, attached to terminal galactose. In further studies we will test the suitability of those lectins for the enrichment of MAP.

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P06

Endothelium-Specific Vitamin D Receptor Ablation Fails to Recapitulate the Cardiovascular Phenotype of Global Vitamin D Receptor Knockout Mice

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Vitamin D deficiency has been associated with cardiovascular dysfunction in several epidemiological studies. In previous experiments in 9-month-old, male, global vitamin D receptor (VDR) knock-out mice fed the so-called rescue diet we showed that vitamin D signaling regulates the expression of endothelial nitric oxide synthase (eNOS). The lower NO bioavailability in global VDR knockout mice was associated with functional and structural changes in the aorta, and with impaired systolic and diastolic left ventricular function. However, a potential caveat in global VDR knockout mice is the widespread expression of the VDR, making it difficult to dissect systemic and tissue-specific effects of vitamin D signaling. Another limitation is that, despite feeding the rescue diet enriched with calcium, phosphorus, and lactose, subtle elevations of serum parathyroid hormone levels remain in aged global VDR knockout animals, which could also influence the cardiovascular phenotype. In an effort to elucidate further the role of vitamin D in regulating endothelial function, we specifically ablated the VDR in vascular endothelial cells, using Cre-lox technology. To this end, we crossed $VDR^{fl/fl}$ mice with Tie2-Cre transgenic mice, specifically expressing Cre in endothelial cells. The specificity of Cre expression was confirmed by crossing the Tie2-Cre mice with $ROSA^{mT/mG}$ reporter mice. Interestingly, echocardiography and cardiac catheterization in 9 – 11-month-old, male and female, endothelium-specific VDR-ablated mice, $VDR^{fl/fl}$, Tie2-Cre, and wild-type control mice on normal diet revealed no difference in arterial stiffness or left ventricular function between the genotypes. Furthermore, collagen content remained unchanged in aortas of endothelium-specific VDR-ablated mice. Thus, specific ablation of the VDR in endothelial cells is not sufficient to recapitulate the cardiovascular phenotype of global VDR knockout mice. This led us to conclude that vitamin D signaling in both, the endothelial and vascular smooth muscle cells, is necessary to exert its protective effect on vascular integrity.

P07

Gel-based proteomics to learn more about effects of the flame retardant HBCD on the liver proteome of female rats

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Hexabromocyclododecane (HBCD) is a brominated flame retardant with low acute toxicity observed in animal experiments, and exposure to it can affect liver function and thyroid hormone status; exact mechanisms are unknown. A gel-based proteomic approach (DIGE) was undertaken in an animal model of eu- and hypothyroid female rats, with low concentration (0-30 mg/kg bw/day) and short term (7 days) exposure via their diet. Alterations in the liver proteome under HBCD exposure were determined in comparison with patterns of control animals of the same thyroid status. This revealed significantly changed abundance of proteins involved in metabolic processes (gluconeogenesis/glycolysis, amino acid metabolism, lipid metabolism), but also in oxidative stress responses, both in eu- and hypothyroid females. Results support a previous hypothesis (based on transcriptomic data) that HBCD influences lipid metabolism, especially in female rats. This is in clear contrast to findings when the same experiment was performed in male rats: here, almost no liver proteome differences were visible, in accordance to previous toxicological reports on sex-dependent differences in susceptibility.

P08

Isolierung von Exosomen aus Blutserum und Zellkulturüberständen zu Diagnostikzwecken beim Hund - Isolation of exosomes from blood serum and cell culture supernatants for diagnostic purposes in dogs

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Moderne medizinische Diagnostik basiert häufig auf molekularbiologischen Analyseverfahren, welche die pathologische Gen-Expression eines Gewebes/Organs charakterisiert. Exosomen sind als extrazelluläre Membranvesikel mit einem Durchmesser von 30-150 nm definiert, die von allen Arten von Zellen stammen können. Sie entstehen durch Endozytose und werden dann mittels Exozytose in den extrazellulären Raum freigesetzt, wo sie in verschiedenen biologischen Flüssigkeiten sowie in Zellkulturmedium gefunden werden. In den letzten Jahren haben Exosomen aufgrund ihres möglichen Einsatzes als Biomarker insbesondere in der Krebsforschung großes wissenschaftliches Interesse erlangt. Es wird hier eine Methode zur Quantifizierung und Identifizierung von Hunde-Exosomen in Blutserum und Zellkulturüberständen unter Verwendung kleiner Volumina (100 µl für Serum bzw. 1 ml für Zellkulturüberstände) vorgestellt. Zur Quantifizierung und Größenbestimmung von Exosomen, die in Serum- und Zellkulturproben enthalten sind, wurden Methoden der Nanopartikel-Tracking-Analyse (NTA), Transmissionselektronenmikroskopie und Immunelektronenmikroskopie eingesetzt. Die detektierten Partikel zeigten sowohl den erwarteten Größenbereich (30-150 nm) als auch die typische Morphologie, welche für Exosomen beschrieben sind. Basierend auf diesem Schnell-Verfahren ist es nun möglich auch aus kleinen Mengen verschiedener Arten von Hundeproben intakte Exosomen effektiv anzureichern. Eine weiterführende spezifische biochemische Charakterisierung exosomaler Biomarker (Nukleinsäuren, Proteine) kann z. B. in der systemischen Tumordiagnostik beim Hund eingesetzt werden.

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P09

The role of DOCK8 and its interaction partners in lymphocytes of ERU cases

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Equine recurrent uveitis (ERU) is one of the most common autoimmune diseases in horses worldwide. It is a dreaded and severe disease in which damage of the retinal tissue is caused by autoreactive T cells. ERU is the only spontaneous animal model representing autoimmune uveitis in humans. During inflammation, peripheral blood leukocytes (PBL) migrate into the inner eye, eventually causing blindness. Trigger mechanisms inducing autoreactive T cells to attack the inner eye are not fully understood to date. Since PBL play a crucial role in the events of this disease, we focused on elucidating changes in protein expression of PBL in diseased horses.

Previous experiments revealed a significantly reduced DOCK8 protein level in PBL of ERU cases. DOCK8 plays an appreciable role in immune response, cell migration and proliferation. We characterized DOCK8 function in healthy and autoimmune cases in depth by elucidating changes in its interaction network. Using interaction proteomics, we identified 442 DOCK8 interacting proteins in both phenotypes. One of the interacting partners of DOCK8, NLRC3, a cytosolic protein and member of the NOD like receptor family, was significantly enriched in PBL of healthy cases. NLRC3 negatively regulates immune response in T cells after contact with pathogens. To reveal further functions of NLRC3, we will examine PBL of both phenotypes, investigating differences in cell proliferation and activation of cytokine pathways after stiumlating PBL with pathogens.

To gain further information in DOCK8 function in disease, we focused on interacting signal transducers. The serine/threonine kinase ILK showed enhanced enrichment in PBL of ERU horses. ILK is involved in important cellular processes such as proliferation and cytokinesis and is attributed to regulate immune cell survival. Comparing ILK protein levels, we found a significant increase of ILK expression in T cells of ERU cases. We presumed that increased ILK expression might affect apoptosis. Examining differences of apoptosis in PBL of both phenotypes using flow cytometery, we found a 2-fold higher apoptosis rate in diseased PBL, whereas necrosis rate was almost identical.

In this study, we aquired novel information about functional changes of DOCK8 and interacting partners in spotaneous autoimmune disease. As ILK and NLRC3 might affect immunological functions of DOCK8 in immune cells, the role of these proteins in ERU merit further investigations.

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P10

Effects of bilateral hydrostatic pressure incubation on the barrier function of mammary epithelial cells

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Outline:
A paracellular sealing effect was observed during milk accumulation in the murine mammary gland, reflected by a changed expression of tight junction proteins in the alveolar epithelial cells (1). To differentiate between effects of hydrostatic pressure and other known influencing factors like milk secretion (2), a modified Ussing chamber had been established to analyze effects of hydrostatic pressure, *in vitro* (3). Using this tool, we tested bilateral hydrostatic pressure effects in dependence of $BaCl_2$ on barrier properties and tight junction proteins in a mammary epithelial cell model.

Methods:
Monolayers of the mammary epithelial cell line HC11 were grown on permeable supports for 7 d, and mounted in modified Ussing chambers. Cells were incubated with a bilateral pressure of 10 kPa for 4 hours. In a parallel approach, 1 mM $BaCl_2$ was added to the bathing solution. Short circuit current (I_{SC}) and transepithelial resistance (R_T) were recorded during incubation, and analyzed compared to controls. After pressure incubation, western blotting and quantification was performed for the tight junction proteins occludin, claudin-3, claudin-4 and ZO-1.

Results:
In both approaches, I_{SC} decreased during bilateral hydrostatic pressure incubation whereas no significant changes were observed for R_T, compared to controls. On molecular level, a reduction claudin-3 and claudin-4 was observed in the setup without $BaCl_2$, compared to controls. Interestingly, with addition of $BaCl_2$ to the bathing solution changes in tight junction composition could not be observed.

Conclusions:
The reduction of I_{SC} and the changes in claudin-3 and claudin-4 could represent cellular adaptive mechanisms. However, the reduction of sealing tight junction proteins may indicate that further maturing processes are needed for HC11 cells to form a physiological barrier, like a longer cultivation time or hormone-induced differentiation.

References
1) Markov AG, Kruglova NM, Fomina YA, Fromm M, Amasheh S (2012) Altered expression of tight junction proteins in mammary epithelium after discontinued suckling in mice. Pflugers Arch. 463: 391-398.
2) Radloff J, Zakrzewski SS, Pieper R, Markov AG, Amasheh S (2017) Porcine milk induces a strengthening of barrier function in porcine jejunal epithelium in vitro. Ann. NY Acad. Sci 1397:110-118.
3) Mießler KS, Vitzthum C, Markov AG, Amasheh S (2017) Basolateral pressure challenges mammary epithelial cell monolayer integrity, *in vitro*. Cytotechnology *in press*.

POSTERS

P11

Characteristics of dipeptide-induced changes in net ion transfer of porcine jejunal and ileal tissues: effects of Resveratrol and the involvement of NHE3 activity

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Resveratrol (RES) is supposed to act as a health promoting substance for various diseases. RES preparations are commercially available and research is going on in order to develop RES enriched food. Therefore it is of importance to gain information about effects of RES on intestinal nutrient and ion transport processes. Na^+-dependent SGLT1-mediated glucose absorption [1] and Na^+-dependent alanine absorption [2] are inhibited after exposure to RES. The aim of the present study was to explore whether short time incubation with RES affects H^+-coupled PEPT1 mediated dipeptide transport and whether this effect may be linked to alterations in the activity of the Na^+/H^+-exchanger 3 (NHE3) that is essential for maintaining the H^+-gradient.

Jejunal and ileal porcine stripped mucosal tissues were incubated in Ussing chambers at three pH levels (5.4, 6.4, 7.4 mucosal) and short circuit currents (I_{sc}) were monitored. Glycyl-L-glutamine (GlyGln, 20mM mucosal) was added after incubation with RES (300µM mucosal, 30 min). In parallel, tissue samples were incubated in organ baths using the same concentrations of RES and tissue samples were used for expression analysis (Western Blot) of NHE3 (total and phosphorylated at Ser552/Ser605) in the apical enterocyte membrane.

Characteristics of GlyGln induced ΔI_{sc} were affected by the intestinal localization and the pH. The apical expression of total NHE3 tended to change accordingly, what may be a reason for changes in peptide induced I_{sc}. RES decreased dipeptide induced ΔI_{sc} in both locations at all pH levels and the extent of inhibition was dependent on both parameters. Rather weak effects of RES on apical NHE3 and NHE3 phosphorylation were observed indicating that other effects of RES are responsible for the inhibition of dipeptide induced ΔI_{sc}.

1. Guschlbauer M., Klinger S., Burmester M., Horn J., Kulling S.E., Breves G. (2013). Comp Biochem Physiol A Mol Integr Physiol. 165:313-318.

2. Klinger S., Breves G. (2016). Tagung der DVG-Fachgruppe "Physiologie und Biochemie" : Berlin, 2016 – Deutsche Veterinärmedizinische Gesellschaft e.V. (Hrsg.) (1. Aufl.) Verlag der DVG Service GmbH, S. 77, ISBN: 978-3-86345-307-7.

P12

Charakterisierung von Proteinprofilen in Uterus-Spülflüssigkeiten von frühträchtigen Stuten mittels 2D-Elektrophorese - Characterization of protein profiles in uterine flushings of early pregnant mares by means of 2D electrophoresis

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Spätestens mit Erreichen des Uteruslumens (ca. Tag 6 nach Ovulation) lösen Pferdeembryonen Veränderungen im Endometrium von Stuten aus. Neben der maternalen Erkennung der Gravidität schaffen diese Umbauvorgänge die Voraussetzungen für die Aufrechterhaltung der Trächtigkeit. Ziel des Projektes war die Gewinnung von Proteinen aus dem Uterussekret von Stuten, um anhand des 2D-Profiles neue Biomarker zu finden, welche später Aussagen bezüglich der Fertilität bzw. des Trächtigkeitsstatus erlauben könnten.

An neun Stuten wurden Uterusspülungen am Tag 7,5 in unterschiedlichen Zyklen durchgeführt: a) Kontrolle - keine Besamung, b) Besamung/Embryo positiv, c) Besamung/Embryo negativ. Für die Uterusspülungen wurden 100 ml Salzlösung (Ringer) eingebracht, über einen Embryonenfilter aufgefangen und für die Analysen vorbereitet. Zudem wurde die Eignung einer weiteren Art der Flüssigkeitsgewinnung aus Pferdeuterus untersucht: Gewinnung des Sekrets mittels Tampons. Es stellte sich heraus, dass bei beiden Verfahren die gewonnenen Proteinkonzentrationen sehr stark schwankten. Nach dem 100ml-Spülverfahren musste eine aufwändige Proteinanreicherung angeschlossen werden, da die Proteinkonzentrationen für weiterführende Analysen zu gering waren (16 - 146 ng/µl). Eine Konzentrierung durch Einsatz von Vivaspin 20 Zentrifugations-Röhrchen 5000 ergab aber eine brauchbare Anreicherung der Proteinkonzentration um das 30-fache (665 - 1130 ng/µl). Die Proben, welche mit der Tampon-Methode genommen wurden, besaßen dagegen eine wesentlich höhere Proteinkonzentration (54,3 bis 79,4 µg/µl), wobei das Volumen teilweise sehr gering war (zwischen 4 - 200 µl). Die Proben aus der Uterus-Spülung wurden mit dem 2D-Clean-up-Kit (GE-Healthcare) für die hochauflösende 2DIGE-Elektrophorese vorbereitet. Nach Fluoreszenz-Markierung wurden die Proteine in der 2D-Elektrophorese getrennt, mittels Laserscanner (Typhoon) detektiert und die Proteinspots mittels DeCyder-Software (GE-Healthcare) ausgewertet. Auf Grundlage dieser 2D-Gele konnten in ersten Experimenten zwischen den Stutengruppen signifikant regulierte Protein-Spots erkannt, isoliert und nachfolgend mittels MS dargestellt werden. Aufgrund der hier vorgestellten methodischen Entwicklungen kann nun eine Validierung dieser uterinen Proteine in einer mit größeren Tierzahlen durchzuführenden zweiten Projektphase bei der Stute durchgeführt werden.

P13

Evidence for the stimulation of multiple conductances by cinnamaldehyde in porcine colon

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Phytonutrients are being investigated for their potential to improve the efficiency of nutrient digestion, but effects on ion transport have rarely been discussed. Colon of pigs was mounted in Ussing chambers at 37°C and gassed with carbogen. Changes in short circuit current (Isc) and tissue conductance (Gt) were used to monitor effects of adding 1 mmol·l^{-1} cinnamaldehyde after replacement of an ion or addition of a blocker. For data evaluation, the changes in Isc and Gt were calculated 15 minutes after the ion replacement or blocker and 15 minutes after addition of cinnamaldehyde. In standard NaCl buffer solution, mean Isc was 9.10 ± 2.12 µA·cm^{-2} and mean Gt 20.34 ± 0.75 mS·cm^{-2}. Addition of 1 mmol·l^{-1} cinnamaldehyde led to significant increases of Isc and Gt by ΔI_{cinn} = 18.35 ± 2.15 µA·cm^{-2} and ΔG_{cinn} = 4.72 ± 0.35 mS·cm^{-2} (both $p < 0.001$). To test for neuronal involvement, 1 mmol·l^{-1} lidocaine was applied, which showed no effect on either ΔI_{cinn} or ΔG_{cinn} ($p > 0.7$). Chloride secretion was inhibited by low chloride solution (9.8 mmol·l^{-1}, both sides), 0.5 mmol·l^{-1} NPPB, 1 mmol·l^{-1} bumetanide, or 0.01 mmol·l^{-1} indometacin. Low chloride or bumetanide had no significant effect on ΔI_{cinn} ($p > 0.1$), while effects of indometacin and NPPB were only partial (62% and 47%, $p < 0.001$). Of these interventions, only low chloride had a small effect on ΔG_{cinn}, which was reduced by 25% ($p = 0.037$). Conversely, bilateral replacement of Na^+ by $NMDG^+$ significantly reduced both ΔI_{cinn} and ΔG_{cinn} (by 86% and 79%, both $p < 0.001$), probably reflecting both effects on Na^+ transport and inhibition of basolateral NKCC. Replacement of mucosal Na^+ reduced ΔG_{cinn} ($p < 0.001$), while ΔI_{cinn} remained unchanged. The ENaC blocker amiloride (1 mmol·l^{-1}) showed no effect on the cinnamaldehyde response. Conversely, 1 mmol·l^{-1} quinidine significantly reduced ΔG_{cinn} ($p < 0.001$). Replacement of mucosal Ca^{2+} by 1 mmol·l^{-1} EGTA increased ΔG_{cinn} ($p < 0.002$). Numerically, ΔI_{cinn} dropped in response to quinidine and rose in response to EGTA, most likely reflecting simultaneous changes of Na^+ absorption and K^+ secretion through the pore of a non-selective cation channel.

The data suggest that roughly half of the cinnamaldehyde response is related to cAMP dependent chloride secretion, most likely via NKCC and CFTR. Quinidine sensitive, amiloride insensitive cation channels may represent the other half of the conductance.

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P14
Glucose transport in Cryptosporidium parvum infected intestinal enterocytes

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C. parvum is a common threat in animal husbandry especially for young calves. In case of infection the parasite is able to cause massive damages in parts of the brush-border membrane which is important for the uptake of nutrients including glucose. It is well known that the uptake of glucose into intestinal epithelial cells can be regulated depending on several wants and conditions. The aim of this project is to examine whether the intestinal epithelium is able to adapt glucose transport mechanisms in the presence of *C. parvum* and secure the glucose uptake nevertheless.

Therefore, a new *C. parvum* infection model was established in IPEC-J2-cells (jejunal porcine enterocytes). To confirm successful infection, hsp70 gene was quantified by real-time PCR. For screening a possible influence on the regulation of glucose uptake, gene expression of the glucose transporters Na-coupled glucose transporter (SGLT) 1, glucose transporter (GLUT) 1 and GLUT 2 in infected and uninfected cells was examined and compared. Furthermore, the protein expression of SGLT 1 was measured in infected and uninfected cells and the actual glucose uptake was measured using radioactively labelled ^{14}C-alpha-methyl-glucose. Here we want to present a new infection model, which enables the illustration of interventions in the regulation of glucose transport caused by parasite infection. First results indicate an adaption of the glucose uptake mechanisms, but rather on the protein than on the gene expression level. This will be elucidated in ongoing investigations.

P15

Activation of hepatic ER-stress in function to severity of experimental polymicrobial sepsis in rats

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The mechanisms underlying multiple organ dysfunction induced by systemic inflammatory response (SIR) are still unclear. Recently, SIR has been reported to induce endoplasmic reticulum (ER) stress. The aim of the present study was to analyze the onset and progression of hepatic ER stress during polymicrobial sepsis in rats. Moderate (16G stent) and more severe (14G stent) sepsis induced by colon ascendens stent peritonitis surgery (CASP) were compared (24h) for the onset of ER stress to respective SHAM animals. Although at 24h after CASP operation no signs of liver injury were apparent, serum levels of IL10 were increased in both CASP groups, TNFa in the 14G group. At this time point TNFa mRNA in the liver was upregulated in both CASP groups, while iNOS and IL-6 mRNA were increased only in the 14G group. ER stress markers (XBP1, GRP78, and CHOP mRNA) were differentially regulated. While GRP78 was increased in both CASP groups, elevated levels of XBP1 mRNA, and its splice variant (sXBP1), were limited to the 14G group. CHOP expression remained unchanged. Using the 14G model, we further analyzed the progression of inflammatory and ER stress markers (up to 96h). GRP78 mRNA preceded (24h, 48h) the transient increase of TNFa, IL-6, and XBP1 expression (48h). At later time points (72h, 96h) no changes were observed. CHOP expression was not changed. Our data show that systemic inflammation affects ER stress branches in an XBP1–dependent and independent manner in 14G and 16G groups, respectively. The absence of induced CHOP expression suggests a successful resolution of ER stress. The pathologic impact of SIR-mediated ER-stress activation in the development of liver failure during abdominal sepsis needs to be further elucidated.

P16

Cardiac arrest leads to delayed neurodegeneration which is associated with decreased heme oxygenase activity in vulnerable brain regions

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Global ischemia as a consequence of ventricular fibrillation cardiac arrest (CA) results in acute neuronal damage followed by a delayed neurodegeneration in particularly vulnerable brain regions, such as hippocampus. It was shown that cell stress and neurodegenerative processes affects the expression of enzymes of the heme degradation pathway, heme oxygenase (HO) and biliverdin reductase (BVR). We therefore questioned whether similar changes can be observed in vulnerable brain regions two weeks after CA and whether the enzyme activities of HO and BVR are affected as well. Brain sections from control (sham) and surviving rats subjected to 6min or 8min of CA and subsequent resuscitation (CA-rats) were analysed two weeks after CA. Motoric and visual cortex, striatum, cerebellum and hippocampus were analysed histologically or for mRNA expression of HO-1, HO-2 and BVR-A and inflammatory markers, tumor necrosis factor (TNF)-alpha receptor 1 by qPCR. Additionally, HO and BVR activities were determined using an enzyme-coupled spectrophotometric assay, yielding bilirubin (BR) in both cases. Rats subjected to CA showed increased hippocampal neuronal loss in the CA1 region which was more consistently found in 8min CA-rats. Rats subjected to CA displayed significantly higher levels for HO-1 mRNA and TNFa-R1 in hippocampal samples, with highest levels in the 8min CA-rats. In CA-rats activity of HO was consistently lower in motoric cortex ($p<0.01$) and hippocampus ($p<0.05$) only. In contrast, neither mRNA nor activities of BVR were affected in any region. Despite the local up-regulation of HO-1 mRNA in hippocampus, a typical sign for the activation of astroglia in response to neuronal damage, the overall capacity to convert heme was decreased, which indicates that CA results in changes similar to those associated with ageing and the onset of neurodegenerative diseases.

P17

Formation of neutrophil extracellular traps during Streptococcus suis meningitis in piglets in vivo: Evasion by nuclease SsnA?

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Bacterial extracellular DNases are described as a main host immune evasion mechanism of various bacteria by degradation of neutrophil extracellular traps (NETs). Besides phagocytosis and degranulation, the formation of neutrophil extracellular traps (NETs) has recently been identified as a fundamental defence mechanism of neutrophils against bacterial infections. NETs, which are released upon contact with pathogens like *S. suis,* consist of nuclear DNA and associated antimicrobial peptides. They are able to immobilize and partially kill the entrapped pathogens. In this study we investigated the role of *S. suis* nuclease SsnA as NET evasion factor *in vitro* and *in vivo* during meningitis in piglets.

First, the nuclease activity and ability to degrade NETs of *S. suis* wildtype and ssnA mutant was tested in vitro. For in vivo experiments, 7 weeks old piglets were intranasally infected with *S. suis* serotype 2 for 2 or 4 days. Then, cerebrospinal fluid (CSF) was collected and analysed for NET formation and bacterial entrapment by NETs.

We were able to show that SsnA contributes to NET degradation and bacterial survival in the presence of NETs *in vitro*. Furthermore, in vivo formation of NETs and bacterial entrapment was confirmed in CSF by immunofluorescence microscopy. Interestingly, despite the activity of *S. suis* nucleases, NET formation in response to *S. suis* wildtype infection was detectable in the CSF of intranasally infected piglets. Based on these results we hypothesize that NETs are stabilized by host factors as the porcine antimicrobial peptide PR-39 against bacterial nuclease degradation in the CSF compartment. In good correlation to these data, animals infected with the *ssnA* mutant developed clinical signs similar as wildtype-infected animals.

In conclusion, *in vivo* data confirmed formation of NETs during *S. suis* meningitis *in vivo*. However, *S. suis* nuclease SsnA is not crucial for virulence under the chosen experimental conditions.

P18

In vivo and in vitro oxygen levels in the cerebrospinal fluid compartment during Streptococcus suis infection

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To search for new therapeutic strategies against meningitis deeper knowledge about the host-pathogen interaction is needed. *Streptococcus (S.) suis* infections lead to meningitis as the bacteria are able to enter the cerebrospinal fluid (CSF) compartment by crossing the blood-CSF-barrier followed by neutrophils as a first immune reaction. Recent investigations showed that neutrophils change their behaviour against pathogens under low oxygen conditions. Therefore we aimed to compare the behaviour of neutrophils in the *S. suis*-infected CSF compartment under atmospheric and physiological oxygen conditions.

In vivo levels of oxygen in the CSF compartment were measured in healthy pigs directly after aspiration into a syringe with a special fluorescence technique (*PreSens*). Additionally oxygen levels in the *S. suis*-infected and uninfected CSF compartment in a blood-CSF barrier model (human choroid plexus papilloma cells) was investigated. These measurements were conducted with specially adapted cell culture well plates (*PreSens*) on an orbital shaker for constant fluid movement. Preliminary results of *in vivo* studies suggest a dissolved oxygen level in healthy pigs between 5 and 9%. The status quo investigations in the cell culture showed a decrease of oxygen in the CSF compartment under normal atmospheric conditions in the incubator (5% CO_2) from about 19% to 8.5% within 2 hours of culture. A further decrease of oxygen down to 3.4% could be observed in case of *in vitro S. suis* infection.

As a future outlook the *in vivo* measurement of oxygen will be performed under defined anaesthetic conditions in healthy pigs and pigs suffering from *S. suis* meningitis. These results are planned to be incorporated to the cell culture model as basis for physiological relevant oxygen conditions during the *in vitro* infection studies.

P19

Interplay between mitochondrial ROS and UPR upon systemic inflammation.

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There is a body of evidence suggesting a link between mitochondrial ROS (mtROS) and the development of ER-stress. We have shown that upon systemic inflammatory response (SIR) ER in liver is delated in close vicinity to mitochondria and peroxisomes, accompanied by ER-stress/unfolded protein response (UPR), suggesting involvement of ROS. Rats injected with LPS showed elevated Xbp1 mRNA levels that reached a steady state by 4 h, while GRP78/CHOP mRNA levels were increased by 8 hours but then dropped by 16 hours, suggesting downregulation of UPR. The aim of this study was to clarify whether mtROS induce ER-stress/UPR. Considering that both mtROS and ER stress result from inflammatory response we exposed rat white blood cells to LPS and collected supernatants containing inflammatory mediators (IM) released 6, 12 and 24 hours after LPS challenge. Rat hepatocytes were incubated with IM for 6 hours and gene expression of inflammatory and ER-stress markers as well as mtROS levels were determined. We observed that inflammatory and ER stress markers were upregulated by all three IM, while mtROS were induced only by 12 and 24 h IM, suggesting that ER stress was not induced by mtROS. To further elucidate the role of mtROS in the onset of UPR we tested the effect of mitochondria targeted antioxidant mitoTEMPO on the response to IM. We observed that mitoTEMPO downregulates inflammatory genes, but upregulates UPR, suggesting that mtROS inhibit UPR and induce unresolved ER-stress as observed in in vivo experiments. These data indicate that mtROS do not induce ER-stress but block the resolution of ER-stress by inhibiting UPR. The pathophysiological significance of these effects needs to be clarified in further in vivo experiments.

P20

Pentraxin 3 is strongly induced in the course of inflammatory conditions in chickens

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Pentraxin 3 (PTX3) is an acute phase protein excessively researched on in mammals. However, until now there is hardly any information about the protein in chickens (chPTX3). Previous work in our lab provided evidence for chPTX3 to be upregulated upon experimental viral and bacterial infections. Here, Next Generation Sequencing shows a strong up-regulation of PTX3 in chickens injected with LPS, which adds up to making it a potential marker for a variety of inflammatory diseases. We further display results of a search on publicly available chicken RNA-Seq data for PTX3 reads, which prove a strong PTX3 up-regulation in a variety of experimentally induced inflammatory conditions. Furthermore, in the course of LPS stimulation and experimental infection, PTX3 expression was analyzed and proved to amplify as compared to untreated controls. This study for the first time provides fundamental information about chPTX3 expression changes upon inflammation and thereby proves its potentially crucial role as a candidate marker for inflammatory conditions and indicator of general health in chicken.

P21

The role for n-3 fatty acids in the modulation of brain-controlled sickness responses during systemic LPS-induced inflammation

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Infection and inflammation are accompanied by an inflammatory response in the brain and by brain controlled sickness symptoms such as fever. During systemic inflammation, these responses are mainly triggered by circulating mediators, which are detected by circumventricular organs like the vascular organ lamina terminalis (OVLT), a critical brain structure for fever induction. Here, we aimed to investigate the role of n-3 fatty acids (FA) on brain inflammation, immune-to-brain communication and fever. Lipopolysaccharide (LPS, 2.5 mg/kg or 50µg/kg) was used to induce systemic inflammation in wild type and fat-1 mice, which are able to produce n-3 FAs endogenously. Locomotor activity, body core temperature, food- and water intake were recorded using a telemetric system. Animals were perfused at specific time points after stimulation and brain sections analysed by matrix assisted laser desorption/ionization mass spectrometry imaging to determine the amount and distribution of phospholipids in the OVLT. Moreover, the inflammatory response in blood, liver and spleen was compared using RT-PCR and bioassays. The high LPS-dose induced short lasting fever in wildtype (WT) mice while fever was absent in fat-1 mice followed by hypothermia in both genotypes. This response was accompanied by significantly lower circulating IL-6 levels in fat-1 mice compared to WT animals. Moreover, we revealed significantly higher LPS-induced expression of TNFα (liver) and nuclear factor interleukin-6 (liver/spleen) in fat-1 mice compared to LPS-stimulated WT mice. When both genotypes were subjected to n-3 FAs deficient diet, these differences disappeared. No differences between genotypes/diets were observed when injecting the low LPS-dose. Moreover, depending on genotypes, diet and treatment, several distinct distribution patterns of phospholipids were observed in the OVLT and surrounding tissue, which are currently semiquantitatively analysed. Interestingly, we showed significantly higher nocturnal locomotor activity of fat-1 mice compared to WT mice. In addition, stress-induced increase of locomotor activity was accompanied by a significantly higher body core temperature in fat-1 mice compared to WT mice suggesting that higher locomotor activity may have contributed to the increase in stress-induced hyperthermia. Overall, the regulation of systemic inflammation by ω-3 fatty acids seems to be complex. Brain inflammatory parameters of fat-1 and WT mice remain to be analysed.

P22

CBD and THC decrease mitochondrial respiration in N18TG2 neuroblastoma cells without influencing cell survival

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High concentrations of phytocannabinoids inhibit the respiratory activity in isolated mitochondria. Nonetheless, it is under debate whether this inhibition has an influence on cell survival since THC and CBD possess neuroprotective capacities. Therefore, we investigated mitochondrial oxygen consumption and cell viability in neuroblastoma cells treated with THC or CBD.

N18TG2 cells were treated for 48hrs with CBD or THC (0.1 to 10 microM) before cell viability was analysed. Activities of enzymes of the electron transport chain were measured in submitochondrial particles. Additionally, Seahorse XF technology, which allows to measure oxygen consumption and glycolysis in intact cells, was performed in cell cultures treated with either cannabinoid (+/- CB1 receptor antagonist SR141716A) for 4hrs.

Neither CBD nor THC reduces cell viability in N18TG2 cells. While the effects of the cannabinoids are alike in submitochondrial particles in which they inhibit enzymes of the electron transport chain, these phytocannabinoids affect mitochondrial respiration differently in neuroblastoma cells. Basal oxygen consumption and ATP production were less reduced after administration of THC (by 16 and 13%) than after treatment with CBD (by 39 and 42%). Neither cannabinoid seems to induce mitochondrial uncoupling. These decreases are not accompanied by a shift to glycolysis and cannot be counteracted by co-treatment with SR141716A.

In N18TG2 cells, THC and CBD inhibit mitochondrial respiration to a differing extend. This may lead to a moderate reduction of the overall cell metabolism that is suggested to be one of the reasons how phytocannabinoids exhibit neuroprotective effects.

P23

Influence of pre-treatment with cisplatin on the reactivity of primary cultures from rat dorsal root ganglia to somatosensory and inflammatory stimuli

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Primary cultures of rat dorsal root ganglia (DRG) mainly consist of neurons, satellite glial cells and a moderate number of ED1 positive microglial cells. Neurons responded to buffer containing a high potassium concentration (50mM KCl) with an increase of intracellular Ca^{2+}-concentration $[Ca^{2+}]_i$. About 70% of these somatosensory neurons also responded to capsaicin, an agonist of the TRPV1-channel with a rise in $[Ca^{2+}]_i$ and could thus be categorized as putative nociceptors, while about 10% of the DRG neurons were responsive to cold and / or the TRPM8-channel agonist menthol and categorized as cold-sensors. Since about 4% of all investigated neurons showed transient Ca^{2+}-signals upon stimulation with the inflammatory mediator prostaglandin E_2 (PGE_2), we investigated further properties of DRG primary cultures under inflammatory conditions, which we simulated by incubation with lipopolysaccharide (LPS, 10 µg/ml). Exposure with LPS for 2 hours resulted in pronounced expression of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL6) in DRG primary cultures as revealed by RT-PCR. In addition, pronounced release of bioactive TNF-α and IL-6 into the supernatants of LPS-stimulated DRG cultures was detected. We further observed a moderate accumulation of the inflammatory transcription factor NF-IL-6 in the nuclei of LPS-exposed neurons and microglial cells. Increased TNF-immunoreactivity was observed in the perinuclear Golgi zone and cytosolic / submembranal vesicles of microglial cells upon stimulation with LPS. In presence of the cytotoxic agent cisplatin (5 or 10 µg/ml), the number of microglial cells was reduced, the growth of satellite glial cells was markedly suppressed, while vitality and stimulus-induced (capsaicin, menthol, histamine, or KCl) Ca^{2+}-signals of DRG-neurons were not impaired. Under these conditions, the LPS-induced production of TNF-α was strongly reduced. Our data suggest a potential role for microglial and satellite glial cells in the initiation of inflammatory processes, which develop in sensory ganglia upon injury or exposure to pathogens. Reduction of the growth of glial cells in primary cultures of DRG by treatment with cisplatin allows improved and longer lasting investigation of neurons upon somatosensory or inflammatory stimulation.

P24

Sepsis is associated with increased circulating intact fibroblast growth factor-23 in mice

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Fibroblast growth factor-23 (Fgf23), a bone-produced hormone, plays a critical role in vitamin D and mineral homeostasis. Human diseases with high intact circulating FGF23 (iFGF23) result in hypophosphatemia and low vitamin D hormone ($1,25(OH)_2D_3$). Furthermore, FGF23 has been linked with inflammatory markers in several inflammatory diseases, suggesting an intricate association between FGF23 and inflammation. Based on these studies and the frequent observation of hypophosphatemia among septic patients, we sought to further elucidate the relationship between Fgf23 and mineral homeostasis in an established murine sepsis model. Sepsis was induced by cecum ligation puncture (CLP) in CD-1 mice of both genders. Baseline CD-1 mice without any surgery were used as controls. Forty-eight hours post-surgery, spontaneous urine was taken, and serum, organs and bones were collected during necropsy. Serum iFgf23 levels increased profoundly in CLP mice compared to baseline animals in a gender-independent manner. Circulating parathyroid hormone (PTH), a well-known stimulator of Fgf23 expression, remained unchanged in CLP mice. Kidney function, as evidenced by serum creatinine and urea levels, was not significantly impaired in CLP mice. These findings show that the CLP-driven increase in iFgf23 serum levels is not caused by increased PTH or a decline in kidney function. Despite the several-fold increase in serum iFGF23 concentrations, we did not observe any significant changes in mineral homeostasis in the blood of CLP animals. However, male CLP mice showed a tendency towards increased urinary phosphate excretion, and female CLP mice exhibited significantly diminished urinary calcium excretion. In conclusion, our data demonstrate a profound increase in serum iFgf23 levels in CLP mice in this well-established sepsis model. It remains to be clarified why the increased iFgf23 was not associated with hypophosphatemia in CLP mice.

P25

Untersuchungen zur Leber-Expression nach Langzeitfütterung von Ratten mit gentechnisch modifiziertem Mais MON810 - Studies on liver expression after long-term feeding of rats with genetically modified maize MON810

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Die Sicherheit gentechnisch modifizierter (GM) Lebens/Futtermittel wird in der Öffentlichkeit kontrovers diskutiert. Die Charakterisierung möglicher Gesundheitsrisiken von GM-Lebens/Futtermitteln wird durch standardisierte 90d-Fütterungsversuche an Ratten durchgeführt. Wir haben in einer Langzeitfütterungsstudie mit GM-Mais (MON810) die Expressionsprofile in Rattenleber erfasst, um mögliche systemische Effekte auf den Leberstoffwechsel nachzuweisen. Nach 1-jähriger Fütterung von Gruppen von jeweils 24 männlichen bzw. weiblichen Ratten mit 33% GM-Maisanteil im Vergleich zu non-GM-Mais wurde Leber-mRNA und -Protein isoliert. Zuerst wurden ausgesuchte mRNA-Leber-Profile (87 Gene korrelierend mit Apotose, *unfolded protein*, NF-κB und DNA-Reparatur) in GM-Mais-gefütterten Ratten im Vergleich zu Kontrolltieren mittels qRT-PCR quantifiziert. Dann wurden mittels *Western Blot* potentiell regulierte Proteine des NF-κB-Stoffwechselwegs näher analysiert. Mehrheitlich NF-κB-relevante Gene zeigten signifikante Konzentrations-Unterschiede zwischen GM und non-GM-gefütterten Ratten. Nach Quantifizierung der korrespondierenden Proteine konnten aber im Gegensatz zur mRNA-Regulation keine signifikanten Unterschiede in der Protein-Expression dargestellt werden. Unterschiede in der mRNA-Expression in Lebern von mit GM-Mais gefütterten Ratten gehen demnach nicht immer mit einer entsprechenden Änderung der Proteinmenge einher. Physiologische Lebereffekte, welche durch eine 33% GM-Mais-Langzeit-Fütterung ausgelöst worden sind, erscheinen in dieser Ratten-Fütterungsstudie unwahrscheinlich. Dies wirft die generelle Frage auf, inwieweit die alleinige Erfassung der mRNA-Konzentrationen in Fütterungsversuchen eine Aussage über mögliche biologische Auswirkungen zulässt, wenn korrespondierende Proteine nicht reguliert erscheinen.

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P26

A new in-vitro model for hepatosteatosis in dairy cows on the way

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Fatty liver occurs in up to 60% of dairy cows around parturition. Until today the underlying cellular mechanisms are not fully understood. So further investigation is needed. With BFH12 we established, to our knowledge, the first fetal bovine hepatocyte-derived cell line This cell line shows a normal hepatic morphology, produces liver-specific metabolites and is sufficiently stable.

In our current study we focus on simulating fatty liver like conditions in BFH12. Palmitic acid (PA, 20 to 80µM), stearic acid (SA, 25 to 100µM) or oleic acid (OA, 50 to 300µM) were added to Williams' Medium E containing 5 % heat-inactivated FBS, 1 % penicillin / streptomycin, 2 mM L-alanyl-L-glutamine, 100 nM dexamethasone and 0.2 U/mL insulin. Initially, we focused on cytotoxic and steatogenic effects of the fatty acids. After 24h incubation we performed a MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay to check cell viability and stained for lipid droplets (LD) with nile red or oil red O. Cellular lipid composition was determined by thin layer and gas chromatography (GC).

The three fatty acids were incorporated into the cells and stored as cytoplasmatic LD. We could show a dose-depend increase in size and number of LD, as well as a decrease in cell survival. OA was less cytotoxic, but more steatogenic than SA and PA. GC analysis revealed that up to 80% of the supplemented fatty acids are incorporated. The fatty acid composition of phospholipids (PL), triacyclglycerols (TAG), non esterified fatty acids and cholesterol esters were different among treatments. OA is the preferred substrate for complex lipid synthesis and leads to a massive increase of TAG and PL. SA is mostly metabolized (β-oxidation, desaturation). PA leads to an increase in PL content. The results underline the potential of BFH12 as an appropriate model of hepatosteatosis.

Comparative investigations on the influence of PA, SA and OA on genes of fatty acid metabolism in BFH12 and fatty liver biopsies are in progress.

P27

Gender differences in bone physiology in aged golden hamsters (Mesocricetus auratus)

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As commonly known, reproductive events bring about both direct and indirect metabolic costs. Traditionally it was assumed that only females bear these costs as they are raising the young and producing the milk. This may be particularly true for a species such as the golden hamster. Due to their solitary behaviour, they raise the pups alone very swiftly and repeatedly during their lifetime. Litter size ranges between 8-14 offspring so females are observed to have very high rates of milk production. We hypothesised that females and males would differ in bone physiology due to reproductive efforts on one hand and also, due to general gender differences. On our poster, we will present bone computer tomography measurements from both cortical and spongy bone with special attention to observed gender differences. We will present an analysis where highly successful mothers raising three litters were compared with non reproductive females.

P28

Pushing the limits of reproduction in mammals: what can be done to maximise milk production?

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Undoubtedly, lactation is the most energy-demanding phase for females where they easily reach up to eight times of their basal metabolism. While the plastic gastrointestinal tract readily supports the several fold food intake to meet the female energy demands, it has become evident over the last couple of years that the high energy turnover rate heats up the female body and brings about a consistently higher body temperature during lactation in several rodent species including mice, golden hamsters, and Mongolian gerbils. Interestingly, the times of hyperthermia and peak energy expenditure and milk production overlap so several measures and approaches all aiming at alleviating the heat stress seem to benefit both milk quantity and female stress. In my presentation that builds up on several series of experiments done in three rodent species, I will summarise the existing data before hypothesising new concepts that result from the endogenous heat dissipation limitation and that aim at facilitating heat loss for the females. Ultimately, a better understanding of energy metabolism during lactation serves both to maximise milk production and juvenile growth but also may improve breeding conditions of laboratory animals and livestock.

P29

Reproduction on a budget: overlapping pregnancy and lactation slows down embryonic development in Mongolian gerbils (Meriones unguiculatus)

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Mongolian Gerbils (*Meriones unguiculatus*) have post partum oestrus. As they are very sociable mammals with strong pair bonds, they reproduce repeatedly every 35-40 days when maintained in harmonic female-male pairs. According to literature their pregnancy lasts 24-26 days, but this is only observed when females were having litters consecutively without males present permanently. Yet, modern laboratory rodent maintenance involves group housing in harmonic families so we tested the impact of overlapping pregnancy and lactation on female and male energy budgets, gestation periods and embryonic development in Mongolian gerbils. Twenty gerbil pairs that were either kept in pairs permanently or separated for the duration of lactation i.e. 21 days, were included into the study. In one group we removed the male on day 2 of lactation to allow copulation after birth and ensuring that the mating took place within 24 hours after birth of the litter. In both groups, we monitored body mass, subcutaneous body temperature, food intake and pup mass over time. Again in both groups, we repeatedly imaged embryonic growth at three time points during each reproductive event. We observed distinct time differences over the course of embryonic development depending on a present or not existing lactation event (i.e. in the primiparous females). From days 16-18 after birth the consecutive pregnancy/litter can be detected by ultrasound in all cases easily. We conclude that slowing down embryonic development in Mongolian Gerbils serves to limit maternal energy expenditure and probably heat production. Future research could to address the role of oxytocin in the slowing down of the development and the degree of maturity of the zygotes from days 1-16 after fertilisation.

P30

Role of ascorbic acid, serum lipids and acetic acid during transdifferentiation of bovine pre-adipocytes to adipocytes.

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The differentiation of pre-adipocytes to adipocytes is depending on many factors. The aim of the present study was to investigate the effects of ascorbic acid (AA), bovine serum lipids (BSL) and acetic acid on the adipogenic potential of bovine pre-adipocytes during differentiation to adipocytes.
Methods: Bovine pre-adipocytes were grown from explant cultures. After 2 d of induction, pre-adipocytes were incubated in media either containing or not containing AA (40 µl/ml), BSL (10 µl/ml) and fetal bovine serum (FBS, 10%) in a three-factorial design. In a second experiment, the effects of glucose concentration (10 or 25 mM) in the copresence of various acetic acid concentrations (0, 10 or 20 mM) were tested. Culture plates had different coatings, i.e., collagen A, gelatin A or poly-L-lysine in comparison to no coating. Adipogenic effects were assessed based on Nile-red staining of cellular non-polar lipids and by quantitative RT-PCR of stem cell and adipocyte markers.
Results: The accumulation of lipid droplets was enhanced ($P < 0.001$) in treatments containing BSL but no FBS compared to all other treatments. DAPI staining, as an indirect measure of cell number, was more intense ($P < 0.001$) in media containing FBS. The mRNA expression of FabP4 increased ($P < 0.001$) in FBS-free media supplemented with BSL, and was additionally promoted by AA. The mRNA expression of CD73, CD90 and CD105 was lowest ($P < 0.001$) in treatments devoid of FBS, except for CD90 where AA prevented such decrease. In the second experiment, the concentration of glucose had no effect on the accumulation of non-polar lipids. The DAPI signal decreased ($P < 0.01$) after 14 d as compared to 7 d; whereas, the accumulation of non-polar lipids was higher ($P < 0.01$) after 14 d with no interaction between these factors and the factors coating and acetic acid concentration. Regarding the latter, a non-significant linear increase in non-polar lipid accumulation was observed with increasing acetic acid concentration. The DAPI signal was lower ($P < 0.001$) in uncoated and collagen A coated plates compared with poly-L-lysine and gelatin A coated plates.
Conclusions: Differentiation of bovine pre-adipocytes is promoted by BSL and acetic acid but inhibited by FBS. Differentiation includes an induction of FabP4 expression, a decrease in CD73, CD105 and, in part, CD90 expression, and is affected by AA. Poly-L-lysine or gelatin A coating improves the retention of differentiating adipocytes on culture plates.

P31

Acid-base variables in acute and chronic form of nontuberculous mycobacterial infection in goats experimentally inoculated with mycobacterium avium subsp. hominissuis and mycobacterium avium subsp. paratuberculosis

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Background / Aim: In the current literature, data assessing the acid-base equilibrium during nontuberculous Mycobacteria (NTM) infection are rare. This study aimed to evaluate changes in acid-base equilibrium in goats infected with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) or *Mycobacterium avium* subsp. *hominissuis* (MAH), respectively.

Study design: In two consecutive long-term studies, overall 91 goats were included (MAP n=48, MAH n=18, controls n=25). In total 2.6 x 10^8 colony forming units (cfu)/goat MAP or 2.1 x 10^{10} cfu/goat MAH were inoculated orally via milk replacer from the 2^{nd} to the 6^{th} week of life (10 times, intervals of 2-3 days). Controls received pure milk replacer. *Post inoculation* (pi) venous blood samples were collected every 4 weeks until 51^{st} week pi. Blood gas analysis, serum biochemical analysis and electrophoresis were performed. The Henderson-Hasselbalch equation and strong ion acid-base variables were calculated.

Results: Two forms of NTM-infection were observed. Fifty percent (9/18) of MAH-infected goats developed an acute severe infection with signs of multi-organ failure, systemic inflammatory response syndrome and sepsis. They died or were euthanized within the first 11 weeks pi. Blood values were characterised by markedly lower concentrations of sodium, calcium, albumin, gamma globulin and total protein associated by a reduced albumin:globulin ratio. Acid-base status indicated alkalosis, but normal base excess and HCO_3^- concentrations. The strong ion model revealed significantly reduced levels of SID (strong ion difference), $A_{tot\ ALB}$ (total plasma concentration of weak non-volatile acids, based on albumin), $A_{tot\ TP}$ (A_{tot} based on total protein) and SIG (strong ion gap).

Nine MAH-infected goats and all MAP-infected animals evolved a chronic form of infection. With the progression of disease, concentrations of gamma globulin and total protein increased while albumin remained lower compared to controls, leading to significantly lower albumin:globulin ratios. Acid-base variables reflected diminutions of AG (anion gap), SIG, $A_{tot\text{-}ALB}$ and increase of $A_{tot\text{-}TP}$.

Conclusions: (i) Acute form of life threatening infection was mainly characterised by alkalosis, significantly lower SID and A_{tot}. (ii) Chronic NTM-infection was dominated by alterations of the blood protein profiles, lower SIG and $A_{tot\text{-}ALB}$. (iii) Only the strong ion variables differentiated alterations in acid-base equilibrium during acute and chronic NTM infection.

P32

HNO as a novel gasotransmitter involved in the regulation of gastrointestinal motility

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An emerging class of regulators of gastrointestinal functions are gaseous molecules, the so-called gasotransmitters. There are two well-established gases with transmitter function in the gut: nitric oxide (NO) and hydrogen sulphide (H_2S). Both induce gastrointestinal smooth muscle relaxation via different mechanisms. While NO activates soluble guanylate cyclase, the production of cGMP and consequently activates protein kinase G (or potentially directly activates K^+ channels), H_2S activates e.g. ATP-sensitive and small conductance Ca^{2+}-dependent K^+ channels. Another gasotransmitter, nitroxyl (HNO), whose actions on gastrointestinal motility shall be investigated in the present study, is getting more attention recently. All what is known about HNO regarding the gastrointestinal tract is that it exerts a prosecretory action, which is strongly dependent on Ca^{2+} and involves Ca^{2+}-dependent and ATP-sensitive K^+ channels and is in part mediated by cyclooxygenase metabolites. Preliminary experiments revealed that an HNO donor, Angeli's salt, evokes intestinal relaxation measured at longitudinal strips from rat ileum and colon. The mechanisms underlying this relaxation are investigated in the present study by isometric muscle contraction measurements.

These experiments show that the HNO-donor Angeli's salt interferes with some pathways activated downstream by acetylcholine, as Rho-kinase blockade tends to inhibit the response to this gasotransmitter. However, blockade of the myosin light-chain phosphatase had no consistent effect on the response to Angeli's salt. In the presence of the soluble guanylate cyclase blocker ODQ, the relaxation evoked by HNO, H_2S and NO is reduced – hinting at an interplay of these three gasotransmitters. Furthermore, imaging experiments at spontaneously contracting smooth muscle cell reaggregates are being performed to further characterize the involved ion conductances and the involvement of typical components of the cGMP signalling pathway. Patch-clamp experiments on myenteric neurons will further eluminate the ionic mechanisms underlying the change in membrane potential induced by HNO on these cells, i.e. the cells responsible for autonomous control of gut motility. With these experiments it is hoped to get a better insight into the regulation of gastrointestinal motility by a new member of the emerging class of gasotransmitters.

P33
The Role of Müller glia cells in Diabetic Retinopathy

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Diabetic Retinopathy (DR) is a severe complication of diabetes mellitus. It is the most common cause of vision loss in adults. The aim of this project is to identify mechanisms of pathogenesis of DR.

For this reason we perform cell culture experiments with cells or retina from healthy, slaughtered pigs. As our studies on INS^{C94Y} transgene pigs have shown recently, eyes from pigs serve as a suitable model for human DR. Beside microvascular anomalies these pigs also develop a central retinal edema, which can be compared to the human macula edema occurring in the course of DR. Diabetic pigs therefore show important, sight- threatening characteristics of human DR.

To induce pathomechanisms of DR *in vitro*, we applied hyperglycemic culture conditions with special interest in the altered protein expression of Müller glia cells. These cells are known to contribute to formation of edema in retinopathies other than DR. Here, we wanted to investigate if they are also involved in genesis of central edema in DR.

To study the influence of hyperglycemic culture conditions on protein expression of Müller glia cells, we isolated primary porcine Müller glia cells and cultivated them under normoglycemic conditions. Subsequently, one group of the cells was treated with a high concentration of glucose in the cell culture medium, another group remained to be cultured under normoglycemic conditions. 24 hours after treatment all expressed proteins were identified and quantified by mass spectrometry based on the LC- MS/MS method. Hyperglycemic treatment revealed a moderate differential protein expression compared to the control group. Ten candidates, which may explain the role of Müller glia cells in genesis of microvasculopathy or edema from a biological point of view, were chosen. To verify these candidates we will apply immunohistological procedures to visualize the differential protein expression. In a next step, we want to examine the influence of hyperclycemic culture conditions on protein expression in organotypic explant cultures of porcine retina. This enables a simulation of the pathological process as close as possible to the *in vivo* situation.

The comparison of the results of this *in vitro* study to our data from the INS^{C94Y} transgene pigs will allow determining the similarities between diabetic specific proteome alterations *in vivo* and those proteome alterations generated by hyperglycemic culture conditions *in vitro.*

P34

Virtuelle Bienenhaltung - ein neues eLearning-Modul für Studierende der Veterinärmedizin / virtual beekeeping - a new eLearning module for veterinary medicine students

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Die Honigbiene ist eines unserer wichtigsten Nutztiere. Die Gesundheit der Honigbiene und die Kontrolle ihrer Produkte unterliegen der Aufsicht von Veterinärmedizinern. In der akademischen Ausbildung der Veterinärmedizin gibt es einen großen Bedarf, die Vermittlung von Grund- und Spezialwissen zum Thema Biene zu optimieren. Zu diesem Zweck wurde an der Freien Universität Berlin ein Internet-gestütztes Lernmodul erarbeitet, um den Studierenden der Veterinärmedizin das Selbststudium zu erleichtern und den vorhandenen Präsenzunterricht zu ergänzen.

Virtuelle Imkerei / virtual beekeeping 1.0: Theorie und Praxis der Bienenhaltung für Veterinäre wurde als eLearning Projekt für Studierende der Veterinärmedizin konzipiert und umfasst unter anderem die Bereiche *Biologie*, *Bienenhaltung* und *Krankheiten* der Honigbiene. Um die praxisrelevanten Aspekte der Imkerei anwenderfreundlich zu vermitteln, wurden audiovisuelle Einheiten sowie interaktive Elemente (z. B. Wissens-Quiz) in das Projekt integriert. Methodisch wurden alle Elemente des Moduls durch Studierende der Veterinärmedizin sowie Experten der Bienenkunde evaluiert. Die Evaluierungsergebnisse sind in die Optimierung der aktuellen Version eingeflossen. Unabhängig von den Jahreszeiten kann das neue Online-Modul die Lehre der Honigbiene über die interne Plattform *Blackboard* an der Freien Universität Berlin praxisorientiert unterstützen. Angehende Veterinärmediziner können angstfrei an die Imkerei und wichtige veterinärmedizinische Routinetätigkeiten, wie Gesundheitskontrollen oder Futterkranzproben, herangeführt werden. Zukünftig sind Erweiterungen dieses Projekts für die Aus- und Weiterbildung von Amtsveterinären sowie eine Öffnung für Multiplikatoren (Schullehrer) vorgesehen. Einblick in das aktuelle online-Modul wird während der Präsentation ermöglicht.

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