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Genetics of affective disorders in relation to tryptophan breakdown

Bengesser S, Lenger M, Maget A, Dalkner N, Hamm C, Fellendorf F, Platzer M, Queissner R, Birner A, Unterweger R, Gostner JM, Fuchs D, Reininghaus EZ.

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Lipid dys homeostasis and the control of bacterial infection

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Infectious diseases can affect lipid homeostasis, reflecting the role of lipids in host pathogen interactions. In case of

infection, HDL-cholesterol and LDL-cholesterol levels decrease, while triglycerides and VLDL increase, causing the so-called lipidemia of sepsis. Although imbalances in cholesterol metabolism lead to high risk of cardiovascular disease, high cholesterol levels in people of European ancestry could suggest an evolutionary advantage of this condition. We hypothesize that alterations of cellular and systemic lipid metabolism alter the course of bacterial infections and the associated host immune responses.

In a murine sepsis model using *Salmonella enterica* serovar Typhimurium (S.t.) we analyzed the bacterial load, the cell count of leukocytes, the gene expression of inflammatory cytokines. Moreover, we determined the lipoprotein profiles of WT and ApoE knockout mice and analyzed the expression of cholesterol metabolism related genes.

The bacterial load in liver and spleen was increased as well as IL6 expression levels. Animals inoculated with S.t. showed elevated levels of total cholesterol compared to vehicle controls. Interestingly, infected animals exhibited increased plasma LDL-cholesterol fractions, which increased gradually with the time post infection. The gene expression of *LDLR*, *Abcb11*, *Cyp7a1*, and *Abcg5* genes was down-regulated, whereas *Abca1* and *Abcg1* expression was slightly increased.

The data obtained suggest that in case of infection the cholesterol metabolism undergoes alterations in order to keep the ApoB containing lipoproteins in circulation, both by up regulating the first steps of the Reverse Cholesterol Transport, and also by down regulating the cholesterol excretion. This data shows that a hyperlipidemic profile could improve the control of bacterial infections.

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Elevated kynurenine levels in diffuse cutaneous and anti-RNA polymerase III positive systemic sclerosis

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Systemic sclerosis (SSc) is a systemic disease characterized by vasculopathy, progressive fibrosis and autoimmune activation. Tryptophan (Trp) metabolism has been linked to altered immune cell function and to malignancy. We have investigated the role of Trp metabolic pathway in SSc measuring serum Trp, Kynurenine (Kyn) and Trp/Kyn ratio in a cohort of 97 SSc patients and 10 healthy controls (HC). Association with disease characteristics was evaluated. We found that Trp levels in SSc patients were significantly lower compared to HCs. We also found that patients with diffuse cutaneous SSc (dcSSc) had lower levels of Trp compared to limited cutaneous (lcSSc). These results were paralleled by higher levels of Kyn found in SSc patients compared to HCs and significantly lower levels in dcSSc compared to lcSSc. The autoantibody profile was also found to be significantly associated with Kyn and Trp levels as anti-RNA-polymerase III (ARA) positive patients were shown to have lower Trp and higher Kyn levels compared with anti-centromere and anti-topoisomerase I positive patients. Moreover, the highest Trp/Kyn was found in ARA+ patients with dcSSc, suggesting that an activation of the Kyn pathway is more specifically associated with this subset of SSc patients. Stability over time makes these markers of Trp metabolism feasible for SSc stratification.

Inflammatory markers in patients with influenza and pneumonia

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Community acquired pneumonia and influenza are two important causes of hospitalization worldwide presenting a major challenge to society. Seasonal influenza epidemics cause up to 5 million severe infections each year and 500000 new cases of pneumonia each year are reported in Germany alone. Several nonspecific markers, namely C-reactive protein (CRP), neopterin, tryptophan, kynurenine, tyrosine and phenylalanine may be of aid when differentiating between viral or bacterial infections. While an elevated concentration of CRP is linked to bacterial infections, an alteration of the other five markers is linked to cell-mediated immunity. Interferon-gamma stimulates macrophages to produce neopterin, increases indoleamine 2,3-dioxygenase (IDO) activity and therefore tryptophan catabolism, and leads to reduced phenylalanine-hydroxylase (PAH) activity resulting in elevated phenylalanine levels.

We determined the CRP to neopterin ratio and examined tryptophan and phenylalanine metabolism to investigate if these parameters allow a better differentiation between viral and bacterial infections.

49 patients diagnosed with influenza or pneumonia at the inpatient ward of the University Hospital for Internal Medicine II between December 2018 and April 2019 were enrolled. In addition to routine laboratory analysis, neopterin, phenylalanine, tyrosine, tryptophan and kynurenine were measured by ELISA and high pressure liquid chromatography. The CRP/neopterin ratio, the kynurenine/tryptophan ratio and the phenylalanine/tyrosine ratio were calculated.

Our study population included 30 pneumonia patients, 10 influenza patients and 9 patients who were diagnosed with both infections. Pneumonia patients showed significantly higher CRP to neopterin ratios compared to the influenza patients. Neopterin levels were elevated (> 10 nmol/L) in all 49 patients and they were significantly higher in influenza patients (median: 60.4 nmol/L) and in patients diagnosed with both, influenza and pneumonia (median: 50.3 nmol/L) than in the pneumonia group (median: 28.6 nmol/L). Kynurenine to tryptophan ratios were higher in patients with influenza and both infections compared to pneumonia patients.

The calculation of the CRP to neopterin ratio is useful to differentiate bacterial and viral infection. Further studies investigating alterations of IDO and PAH activity in viral infections might be interesting.

Iron metabolism is a regulator of whole body cholesterol homeostasis

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Dyshomeostasis of iron metabolism has been linked to the development of cardiovascular disease. However, the underlying mechanisms remain poorly understood.

By combining data from a GWAS screening in >188,000 individuals of European ancestry, CRISPR/Cas9 genome editing, and functional validation studies in mice, we identified iron metabolism as an important regulator of cholesterol homeostasis.

Iron overload of dyslipidemic mice strongly reduced plasma low density lipoprotein cholesterol (LDL-C) levels, thereby inhibiting atherosclerosis development.

Mechanistically, we identified a novel iron – related regulator of LDL receptor expression in hepatocytes, whereas iron promoted the transfer of cholesterol from Kupffer cells (KCs) to hepatocytes in an Abca1-dependent fashion. Importantly, we characterized KCs as a gatekeeper of lipid homeostasis by controlling hepatic removal of LDL-C.

Our results define iron metabolism as conserved regulator of cholesterol homeostasis and identify KCs as promising therapeutic targets to treat dyslipidemia in humans.

Tetrahydrobiopterin-dependent regulation of immunometabolism in inflammatory macrophages by nitric oxide

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Classical activation of macrophages elicits the expression of inducible nitric oxide synthase (iNOS), generating large amounts of NO and inhibiting mitochondrial respiration. Upregulation of glycolysis and a disrupted tricarboxylic acid (TCA) cycle underpin this switch to a pro-inflammatory phenotype. We show that the NOS cofactor tetrahydrobiopterin (BH4) modulates interleukin 1 beta production and key aspects of metabolic remodelling in activated murine macrophages via NO production. Using a ‘multi-omic’ approach in two complementary genetic mouse models, we reveal that NO modulates levels of the essential TCA cycle metabolites citrate and succinate, as well as the inflammatory mediator itaconate. Furthermore, NO-deficient cells upregulate glycolysis despite changes in the abundance of glycolytic intermediates and proteins involved in glucose metabolism. Studies correlating these metabolic changes with altered BH4-dependent redox signalling will also be presented. Our findings reveal a fundamental role for tetrahydrobiopterin and iNOS-derived NO in regulating metabolic remodelling and cytokine production in the pro-inflammatory macrophage.

Plasma kynurenine levels and kynurenine/tryptophan ratios are associated with colorectal cancer in the Austrian “Salzburg Colon Cancer Prevention Initiative” study

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Colorectal cancer (CRC) is a frequent type of malignancy and an important cause of cancer-related deaths on a global level. Early and accurate diagnosis and staging of CRC are crucial to improve prognosis, and largely depends on invasive approaches. CRC progression probably associates with metabolic alterations and altered metabolite profiles. Less-invasive approaches such as serum metabolomics may assist in appropriate

molecular staging of the disease. 82 participants of the SAKKOPI (Salzburg Colon Cancer Prevention Initiative) program were allocated to one of three groups according to the results of the screening colonoscopy: Advanced adenoma (AA, $n = 28$), colorectal carcinoma (CRC, $n = 18$), control (CTRL, $n = 36$). Serum samples were analyzed for routine clinical parameters and for 188 endogenous metabolites by LC-MS/MS, using the Absolute IDQ p180 kit (BIOCRATES Life Sciences, Innsbruck). Overall, 50 metabolic features were different between the adenoma and carcinoma group. Total dimethylarginine (TDMA) serum concentration was significantly elevated in the carcinoma group compared to healthy controls (0.83 vs. 1.01; $p < 0.001$) and the adenoma group (0.89 vs. 1.01; $p = 0.007$). The Kynurenine/Tryptophan ratio was significantly higher in the carcinoma group than in the healthy control group (0.041 vs. 0.060; $p = 0.038$). Our study detected metabolites that clearly differentiate colorectal carcinoma from advanced adenoma patients. Such metabolites may be targeted in mechanistic studies to understand underlying biochemical pathways and could serve to improve diagnosis and staging of CRC. The role of TDMA serum concentration and the Kynurenine/Tryptophan ratio as a potential biomarker for the identification of colorectal carcinoma deserves further investigations.

Characterisation and exploitation of Group A streptococcal anti-chemotactic proteases

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The Group A Streptococcus, *Streptococcus pyogenes*, is a major human pathogen, distinguished by an exquisite ability to escape the innate immune response and initiate systemic and severe infections. Central to its pathogenesis are two bacterial surface-associated serine proteases, SpyCEP and ScpA that inactivate specific neutrophil chemotactic signalling pathways. SpyCEP (*S. pyogenes* cell envelope proteinase) cleaves and inactivates CXC chemokines notably CXCL8, while C5a peptidase (ScpA) cleaves and inactivates the anaphylatoxins C5a and C3a.

The site of cleavage by both enzymes is within a C-terminal α -helix, truncating the substrates by 6-13 amino acids. The rationale for ScpA cleavage of the C5a C-terminus is obvious, since the C-terminal arginine of C5a plays a key role in C5a receptor activation with endogenous carboxypeptidases generating the less potent C5adesArg during resolution. In contrast, the N-terminus, rather than the C-terminus of CXCL8 is noted for playing a pivotal role in chemokine receptor activation. Using transfectants we showed that SpyCEP-cleaved CXCL8 was unable to bind or activate CXCR1 or CXCR2 [1]. Studies by others have shown that monomeric and dimeric CXCL8 play complimentary roles in neutrophil recruitment *in vivo*, by facilitating the formation of an immobilized (haptotactic) gradient of CXCL8 in tissues. Multimerization of CXCL8 is aided by binding to glycosaminoglycans (GAGs) with the C-terminus playing a crucial role. Cleavage of CXCL8 by SpyCEP rendered the chemokine unable to bind to heparin and resulted in the meandering migration of neutrophils [1]. Thus, we postulate that CXCL8 binding to neutrophil cell-surface GAGs is a critical first step for receptor activation and CXCL8 function. This suggests novel strategies to target excessive neutrophil recruitment in disease and highlights SpyCEP as a promising target for vaccine development.

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Gammaproteobacteria dysbiosis in the very low birth weight infant: associations and outcomes

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The microbiomes of 83 preterm very-low-birth-weight (VLBW) infants and clinical covariates were analyzed weekly over the course of their initial neonatal intensive care unit (NICU) stay. Microbial diversity increased with age and was significantly correlated with weight gain and percentage of the mother's own milk. Infants who received antibiotics during their NICU stay had significantly lower alpha diversity than those who did not. Using the metagenomics inference tool Piphillin we determined predicted metabolic pathways based on abundances, using the KEGG database and found that the tryptophan, tyrosine and phenylalanine biosynthesis was lower in preterm infants.

Of those in the cohort, 25 were followed into childhood. Alpha diversity significantly increased between NICU discharge and age 2 years and between age 2 years and age 4 years, while the microbial alpha diversity of 4-year-old children was not significantly different from that of mothers. Microbial beta diversity results were significantly different between the time of the NICU stay and all other time points (for children who were 2 or 4 years old and mothers when their children were 2 or 4 years old). There were relationships between NICU gut microbiome diversity and abundances of ASVs with 2 and 4 years old children's behavior and neurodevelopment.

Tryptophan metabolites and plasma cytokines across pregnancy and the postpartum in *Toxoplasma gondii* positive and negative Hispanic women

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Toxoplasma gondii is a worldwide infection that exists after the acute phase as a chronic, usually latent, encysted brain and muscle infection. Immune deficiency can reactivate the parasite, and the risk for reactivation during pregnancy is unknown. Hispanic women have a higher seroprevalence and may be infected with a more virulent serotype. In an ongoing study of Hispanic women, studied through pregnancy and the postpartum, we are collecting immune, metabolic, and psychosocial variables in women positive for *T. gondii* IgG, compared to a negative control group. Women are screened in the first trimester for *T. gondii* IgG (mean of 12 weeks) and then followed with three additional visits (mean weeks 20, 27, and 33) through their pregnancy and early postpartum. Tryptophan catabolites and a panel of cytokines are measured at each visit. Participants complete a demographic scale, the MOS (social support) the Edinburgh (depression) and the Perceived Stress scale. Longitudinal metabolic and immune measures were examined with mixed models that examined effects of time and *T. gondii* status. Forecasting models using cross correlations were used to examine

correlation of metabolic and immune parameters over time. 345 women have currently been screened, with a 21.5% prevalence of positive *T. gondii* IgG titers. One third of the *T. gondii* positive women experienced preterm birth, while the negative control group had a 9% incidence. The titers for *T. gondii* IgG declined over the pregnancy. A metabolic panel measuring tryptophan catabolism at intake into the study showed dropping levels of tryptophan ($F=14.28$, $p < 0.001$) and higher kynurenine ($F=20.5$, $p < 0.001$). *T. gondii* status did not contribute to metabolic markers. The phenylalanine/tyrosine ratio ($F=16.4$, $p < 0.001$) and nitrite levels ($F=11.9$, $p < 0.001$) rose across pregnancy, dropping in the postpartum.

Cytokines also changed across pregnancy and the postpartum, with most cytokines rising. However IFN- γ , IL-10, and IL-12 were lower across time in the *T. gondii* positive women. Cross correlations of neopterin with IFN- γ , IL-6 and TNF- α all showed contemporaneous correlations.

These are early results in a study that will ultimately enroll 200 *T. gondii* positive and 200 *T. gondii* negative Hispanic pregnant women. The data suggest that pregnancy is associated with nitrosative, metabolic, and inflammatory stress. Current work is exploring these variables in preterm birth.

The MESI-STRAT consortium: exploring the crosstalk of metabolism and signaling for breast cancer patient stratification and targeted therapy design

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on behalf of the MESI-STRAT consortium (<https://mesistrat.eu/>)

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Breast cancer is a complex disease with high prevalence in Western countries. 80% of the patients suffer from estrogen receptor (ER)-positive tumours and are treated with endocrine therapies targeting the ER. As the ER is a signalling molecule, research and clinical trials on therapy resistance focus so far mainly on the crosstalk of the ER with other oncogenic signalling networks. As a result, compounds inhibiting signaling kinases are in clinical use and trials as second line therapies for ER-positive breast cancer. However, the success of these interventions remains limited as they are often not curative.

In contrast to kinase signalling, only little is known about the contribution of tumour metabolism to endocrine therapy resistance. Focusing on tryptophan and its metabolites, MESI-STRAT explores the interplay of amino acid metabolism with signalling networks to identify marker metabolites that predict relapse and guide targeted interventions at an early stage. Towards this goal, a pan-European team of oncologists, modelers, bioinformaticians and experimentalists develops new computational models in combination with network analyses and pharmacogenomics, to integrate multi-omics data and explore metabolic and signalling networks in breast cancer.

Control of oncogenic signaling by tryptophan

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Tryptophan (Trp) metabolism is increasingly being recognized to enhance tumor cell malignancy. Glioblastoma

are a devastating tumor entity with few treatment options and an average survival of less than two years. Thus, there is a high medical need to develop new treatment strategies for glioblastoma patients. Glioblastoma often exhibit enhanced levels of the Trp-degrading enzyme TDO2 (Trp 2,3-dioxygenase), resulting not only in increased amounts of oncogenic Trp metabolites, but also in the depletion of the essential amino acid Trp. We propose that oncogenic signaling networks play an important role in mediating the pro-tumorigenic effects of Trp. Using cell biology, biochemical, and systems approaches, we explore novel links between oncogenic signaling and Trp catabolic networks and the cancer-relevant outcomes of this interplay.

10 years Biopsychronology...the past, the present and the future

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The development of new methods to monitor the status of a graft prior to transplantation are highly desirable to avoid unnecessary surgical interventions and follow-up treatments and to optimize the clinical outcome as delayed graft function may lead to costly and lengthy follow-up treatments or even organ loss.

For this purpose we developed a method, which combines the use of biopsies, the staining of living cells with dyes suitable to monitor mitochondrial status/cellular integrity and real time live confocal imaging. We used the following stains: propidium iodide (PI), which is only taken up by nuclei of dead cells, Syto16, which stains nuclei in dead and living cells, and fluorescently labelled wheat germ agglutinin conjugate (WGA), which stains cell membranes as well as matrix components and thus can be used to monitor the intactness of the cells and tissues.

Our results demonstrate the principal usefulness of this method to monitor organ damage. Kidney, liver and heart tissue may be analysed in real time. Surprisingly, even despite immediate processing of the biopsies, heterogeneity in the staining pattern was observed with viable and non-viable cells present. This is especially the case in heart biopsies.

Our findings demonstrate that real time live confocal microscopy requires minimal time for sample preparation and is well suitable to record organ viability. We advertise its use for the *ex vivo* assessment of organ function in humans prior to transplantation, but also in the setting of experimental transplantations in animal models. Since biopsies can be cultured *in vitro*, this approach may also be helpful in the *in vitro* pre-testing of therapeutic interventions.

Immune-mediated changes in tryptophan and phenylalanine metabolism in patients with pneumonia

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Pneumonia is a leading cause for morbidity and mortality worldwide and is also a frequent cause for hospitalization and ICU-admission [1,2]. During infection and inflammatory conditions, an increase in enzyme activity of indoleamine 2,3-dioxygenase (IDO) leads to an increased breakdown of tryptophan (TRP) to kynurenine (KYN) as part of the immune defense [3-9]. On the other hand, cellular immune activation leads to upregulation of neopterin production whereas a reduced conversion of phenylalanine (PHE) to tyrosine (TYR) has been described [10-12]. The aim of our study was to investigate alterations in tryptophan and phenylalanine metabolism in adult patients with pneumonia.

We measured levels of TRP, KYN, PHE and TYR by high-performance liquid chromatography in patients diagnosed with pneumonia, shortly after admission. Measurements were compared to a cohort of 100 healthy controls. Also symptoms, vital parameters on admission, comorbidities, short-term adverse outcome (30-day-mortality, ICU-admission) and detection of a pathogen were assessed.

Overall, 57 patients with pneumonia were included. Patients had significantly lower levels of TRP ($p<0.001$) and TYR ($p<0.001$) and higher levels of KYN ($p<0.001$) and PHE ($p<0.001$) compared to healthy controls. Also, neopterin levels were significantly higher in patients with pneumonia ($p<0.001$). KYN/TRP ratio, PHE and PHE/TYR

ratio showed a positive correlation with CRP as a marker for inflammation, whereas TRP and TYR showed negative correlations. KYN, KYN/TRP ratio, PHE and PHE/TYR ratio also showed positive correlations with neopterin as a marker for immune activation. Patients with detection of *Legionella pneumophila* antigen had significantly higher neopterin levels than patients with *Chlamydia pneumonia* antibodies ($p=0.031$) and higher PHE levels than patients with pneumococcus detection ($p=0.008$).

Patients with pneumonia seem to have significant changes in levels of TRP, KYN, PHE, TYR and neopterin. Also, an association of acute phase inflammation and immune activation parameters with alterations in TRP/KYN- metabolism and PHE/TYR- metabolism was found. However, further prospective, larger-scale studies are needed to determine effects on clinical outcome, disease severity and therapeutic implications.

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Modulation of immunobiochemical pathways by myrobalane fruit extracts *in vitro*

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The fruits of *Phyllanthus emblica*, *Terminalia chebula* and *Terminalia bellirica*, commonly termed as myrobalane fruits, are frequently used in traditional medicine. Different activities have been reported for Triphala, a herbal remedy containing all three fruits, ranging from antidiabetic, cardiovascular, anti-inflammatory, anti-oxidative to hepatoprotective effects, however mode(s) of action are not understood on a molecular level [1,2].

An *in vitro* study utilizing different human cell models revealed interesting effects on inflammation associated pathways of the individual fruit extracts as well as a mixture thereof. Myrobalane fruit extracts exhibited significant and dose-dependent anti-oxidative and radical-scavenging properties, they reduced interferon- γ dependent tryptophan breakdown as well as the formation of the oxidative stress marker neopterin in lipopolysaccharide-stimulated THP-1 cells. Moreover, nuclear factor- κ B (NF- κ B) activity was affected. Antioxidant response element (ARE)-dependent gene expression, which plays an important role in inflammation and stress response, was shown to be induced synergistically by the three myrobalane fruits in HepG2 liver cells.

In summary, data confirm the potential of the three individual plant extracts to interfere with the above-mentioned pathways, together with synergistic effects of the multicomponent mixture, which might help to understand the mode of action of such complex preparations.

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An investigation on the interactions between occupants and the microbiome of the built environment

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Individuals in Westernized societies spend 90% of their times indoors, exposed to a set of biological agents non-similar to those in a natural environment. In the last century, indoor biological agents of research interest had been limited to allergens, mold, and infectious disease transmission. Yet in the past decade, with an advancement in molecular sequencing and bioinformatics, detailed microbiome studies have been undertaken to explore the full microbiome of the built environment. These studies have provided a broad level of understanding on how occupants and the built environment share an interaction at a molecular level. The microbiome found in the indoor environment has been assessed through air collection, surface swabbing, vacuumed dust collection, settled dust collection, and heating, ventilation and air conditioning (HVAC) filter retrieval. Each method has its advantages and limitations. Independently of sampling method chosen, the microbiome of the built environment consists of biological agents from a mixture of those from outdoors, humans, and pets. The heterogeneous nature of all three factors can be confounded by lifestyle, architecture and external environmental exposures.

Therefore, we conducted a longitudinal study of United States Air Force Academy cadets ($n = 34$), which have substantial homogeneity in lifestyle, diet, and age, all factors that influence the human microbiome. We characterized bacterial communities associated with: (1) skin and gut samples from roommate pairs; (2) four built environment sample locations inside the pairs' dormitory rooms; (3) four built environment sample locations within shared spaces in the dormitory; and, (4) room matched outdoor samples from the window ledge of their rooms.

Linear convergence of microbial composition and structure was observed between an occupant's skin and the dormitory surfaces that were only used by that occupant (i.e., desk). Conversely, bacterial community beta diversity (weighted Unifrac) convergence between the skin of both roommates and the shared dormitory floor between the two cadet's beds was not seen across the entire study population. The sampling period included two semester breaks in which the occupants vacated their rooms; upon their return the beta diversity similarity between their skin and the surfaces had significantly decreased compared to before the break ($p < 0.05$). There was no apparent convergence between the gut and building microbiota, with the exception of communal bathroom

door-handles, which suggests that neither co-occupancy, diet or lifestyle homogenization had a significant impact on gut microbiome similarity between these cadets over the observed time frame. As a result, predictive classifier models were able to identify an individual more accurately based on the gut microbiota (74%) compared to skin (51%). To the best of our knowledge, this is the first study to show an increase in skin microbial similarity of two individuals who start living together for the first time, and who are not genetically related or romantically involved. Cohabitation was significantly associated with increased skin microbiota similarity, but did not significantly influence the gut microbiota. Following a departure from the occupied space of several weeks, the skin microbiota, but not the gut microbiota, showed a significant reduction in similarity relative to the building. Overall, longitudinal observation of these dynamics enables us to dissect the influence of occupation, diet and lifestyle factors on occupant and built environment microbial ecology.

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Acute and chronic mental stress both influence levels of neurotransmitter precursor amino acids

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Acute and chronic mental stress are linked to somatic and psychiatric morbidity, however, the neurobiological pathways of these associations are still not fully elucidated. Mental stress is known to be immunomodulatory, which is one of the basic concepts of psychoneuroimmunology. The aim of the current study was to investigate whether acute and chronic mental stress would influence circulating levels of neurotransmitter precursor amino acid levels.

Methods: 55 healthy subjects were subjected to an acute mental stress test (factor: acute mental stress), once in the presence and once in the absence of a major university exam preparation period (factor: chronic mental stress). Covariates were also included in the mixed

linear model analysis. Psychometric measurements of acute and chronic mental stress were collected, including visual analogues scales, the perceived stress scale, the Trier inventory for chronic stress (TICS). Depressive and anxiety symptoms were also assessed. C-reactive protein (CRP), neopterin, kynurenine/tryptophan (KYN/TRP) and phenylalanine/tyrosine (PHE/TYR) were analysed by HPLC or ELISA prior to and at 0, 30 and 60 minutes following the acute mental stress test.

Results: Neopterin was increased in the chronic stress condition. KYN/TRP was influenced by the factor acute mental stress (increased KYN/TRP concentrations due to acute mental stress), no influence of the factor chronic mental stress or interaction was found. PHE/TYR was influenced by the factor acute mental stress (increased PHE/TYR concentrations due to acute mental stress) as well as by chronic mental stress (decreased concentrations due to chronic mental stress). Interactions were not significant. KYN/TRP correlated with state trait anxiety inventory state values ($r=0.178$ $p<0.001$) while PHE/TYR correlated negatively with stress parameters (TICS chronic stress: $r=-0.112$, $p=0.021$; PSS: $r=-0.099$, $p=0.044$).

Discussion: The increase of KYN/TRP and PHE/TYR concentrations due to acute mental stress could relate to inflammatory processes and might be responsible in the pathophysiology of acute stress-related coronary events. The influence of chronic mental stress with a PHE/TYR decrease could relate to an exhaustion of some aspects of pro-inflammatory activity during prolonged exposures to chronic mental stress. This exhaustion of the pro-inflammatory system has been demonstrated in a similar chronic stress paradigm for the response to vaccinations.

Phylloxanthobilins as overlooked components of medicinal plants - investigations on bioactivities

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In the course of the last three decades, the linear tetrapyrrolic products of chlorophyll breakdown, named phyllobilins, have come to our attention. Considered 'mere' left overs of a controlled biological chlorophyll detoxification at first, phyllobilins are now believed a new opportunity for discovering relevant bioactivities [1]. Phyllobilins are

abundant in leaves, fruit and in some vegetables [2,3]. Recently, yellow phyllobilins, the phylloxanthobilins, were shown to occur in unprecedented abundance and diversity in *Echinacea purpurea*, a plant with undoubted high importance in phytomedicine. Phylloxanthobilins were even discovered in fresh green leaves - without any 'obvious' chlorophyll breakdown [4]. Many different pharmacological activities have been demonstrated for *Echinacea purpurea*, nevertheless, neither a single compound nor a compound class has been identified that can account for all of the efficacies. Caffeic acid derivatives are considered a relevant class of constituents with anti-oxidative activity. We are able to demonstrate a high anti-oxidant potential of the phylloxanthobilins isolated from *Echinacea*, superior to caffeic acid in *in vitro* experiments and comparable to caffeic acid in cellular approaches. Using the PBMC model, anti-inflammatory properties were demonstrated for a phylloxanthobilin [5], indicating a role in immunomodulation for these natural products. Future studies aim at investigating the contribution of phylloxanthobilins to beneficial effects of medicinal plant extracts.

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Fatigue, depression and quality of life in patients with post-infectious fatigue - a prospective, double-blind, placebo-controlled pilot study

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A significant percentage of patients suffers from post-infectious fatigue, especially after viral infections. Currently, no evidence-based treatment is available.

In our prospective, double-blind, placebo-controlled pilot study, we investigate scores of quality of life (SF-36), depression (Beck's Depression Inventory II (BDI-II),

fatigue (Fatigue Severity Scale (FSS)) and the immune status in patients with post-infectious fatigue. We want to find out whether treatment with a multi-strain probiotic can improve patients' fatigue, quality of life and digestion. Furthermore, we also determine immune-mediated alterations of tryptophan and phenylalanine metabolism and their relationship with neuro-psychiatric symptoms and gastrointestinal dysfunction.

Up to now, 50 patients have been included. At baseline, 26 patients suffered from depression, 72% of them had severe fatigue (mean FSS 5.32) and reported about a significantly impaired role function due to physical and emotional problems. 36% of the participants had neopterin values above 8.7 nmol/L (95th percentile). Interestingly, most laboratory parameters were within reference ranges in our patients.

Patients with post-infectious fatigue often suffer from depression and impairment of their physical and emotional health. We try to evaluate if probiotics might be a treatment option for these patients.

Serum Neopterin Levels and IDO Activity as possible prognostic markers of Hepatitis B

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Hepatitis B is a worldwide health concern caused by Hepatitis B Virus that has variable clinical consequences such as fulminant, acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma as well as asymptomatic carrier state. Chronic Hepatitis B is one of the major reason for liver related mortality and morbidity. The aim of our study was to evaluate the possible relationship between the immune system activation and prognosis of Hepatitis B by monitoring tryptophan degradation and serum neopterin levels in patients with Hepatitis B virus (HBV). 110 patients (60 males, 50 females) and 23 healthy subjects (11 males, 12 females) as a control group were included in our study. Hepatitis biomarkers were determined in the hospital biochemistry

laboratory. Serum kynurenine (Kyn) and tryptophan (Trp) levels were measured by high performance liquid chromatography and Kyn/Trp ratio is used to express the indoleamine 2,3-dioxygenase (IDO) activity. Competitive ELISA assay method was used for the determination of neopterin levels. Comparison of neopterin and Kyn/Trp levels of patients and control group has shown that both markers are significantly higher in the patients with HBV infection. There was also positive correlation between neopterin level and Kyn/Trp ratio in patients. Neopterin levels and IDO activity was remarkably higher in the patients with histological activity index (HAI) ≥ 4 compared to the patients with HAI < 4 . Moreover, a significant correlation was found between neopterin levels and HAI. There was a strong negative correlation between albumin levels and IDO activity in HBV patients. These findings suggest that Trp degradation results from IFN-g-induced IDO activation and decreased albumin synthesis may result in increased Trp degradation over the Kyn pathway. In conclusion, based on the study result, serum neopterin levels and IDO activity suggested to provide additional immunological information for monitoring liver histological activity and can be used as a prognostic markers in HBV disease.

Interpreting biological phospholipidomics data by application of artificial neuronal networks

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Most living organisms rely on highly regulated membrane lipid architectures. These membranes consist of proteins, cholesterol, and a variety of lipids defining the physicochemical membrane properties by bilayer formation. In many diseases with an aberrant lipid metabolism, where the phenotype is already well described, the underlying pathomechanisms are still unknown. Research in this area of interest is further convoluted by the fact, that even in a healthy organism various tissues present with diverging membrane lipid

states. Today we still know little about the factors responsible for exactly regulating lipid homeostasis.

Therefore, to investigate complex research questions with only vague background information, we performed LC-MS/MS lipidomics in a series of different mouse tissues. The results were then used to train a supervised machine learning algorithm to model lipid landscapes. We here report a strong agreement between measured and predicted data that strengthened our hypothesis that acyl residue availability in cellular phospholipids plays a central role in defining lipid diversity in mitochondrial membrane lipids.

Inflammation coincides with enhanced vitamin D metabolism in patients with coronary artery disease and is associated with a worse outcome

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Cellular immune activation and alterations of vitamin D metabolism like elevated C-terminal (Ct)-FGF-23 levels have been described in patients with cardiovascular disease. The aim of our study was to investigate the role of inflammation in patients with coronary artery disease and their relationship with vitamin D metabolism and the outcome of patients.

In 2079 patients from the Ludwigshafen risk and Cardiovascular Health study (LURIC) parameters of inflammation (CRP, neopterin, leukocyte counts) and vitamin D metabolism (vitamin D, Ct-FGF-34, calcium, phosphate, PTH, klotho) were determined and correlated with each other as well as with the outcome of patients (follow-up for 10 years).

Patients with increased neopterin and/or CRP concentrations had a worse outcome compared to patients with normal levels of inflammatory markers. Patients with both parameters elevated had the worst outcome. Higher FGF-concentrations were also associated with an impaired survival of patients. Higher Ct-FGF-23 concentrations were

found in patients with higher inflammatory markers and low vitamin D levels. Patients with high neopterin and high FGF-23 levels had a significantly worse outcome than those with low neopterin and Ct-FGF-23 levels.

The findings of our study indicate that inflammation coincides with disturbances of vitamin D metabolism in patients with CAD, and that the elevation of neopterin and Ct-FGF-23 concentrations are predictive for a worse outcome of patients.

The alkylglycerol monooxygenase deficient mouse: gene structure and physiology

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Alkylglycerol monooxygenase (Agmo) is a highly hydrophobic transmembrane enzyme dependent on the vitamin-like cofactor tetrahydrobiopterin. It is the only enzyme described yet capable of cleaving the saturated ether bond of alkylglycerols generating free fatty aldehydes and glycerol derivatives. In 2010, our laboratory discovered the Agmo gene [1] and recent publications indicate a role of Agmo in lipid metabolism of murine RAW264.7 macrophage-like cells [2] pointing to a potential implication in immunology together with other reports on genetic predisposition of humans for leishmaniosis and tuberculosis. Furthermore, mice with reduced tetrahydrobiopterin levels appear to have alterations in their blood lipid concentrations as well as in their body fat distribution [3]. Nevertheless, little is known about the physiological and pathophysiological role of Agmo.

In order to study Agmo physiology, we have established an Agmo knockout in C57bl/6J mice starting from EUCOMM stem cells, which harbor a Agmo conditional knockout first allele. In this knockout first allele expression of the reporter lacZ is linked to the Agmo promoter via a strong splice acceptor site after exon 1 and insertion of

this cassette disrupts translation of a functional Agmo protein. Crossing these mice with a flippase deleter mouse yields the conditional allele tm1B, which restores normal and undisrupted Agmo function. Further mating steps with a ubi-cre deleter mouse deletes exon 2 in early embryogenesis causing a frameshift mutation that leads to the loss of Agmo.

Standard procedures for genotyping Agmo knockout mice were not applicable because of a duplication event in the Agmo gene during homologous recombination, which we were able to characterize by nanopore sequencing. Due to this integration event, genotyping has to be performed by counting knockout alleles by quantitative PCR. Having established the genotypes, we were able to analyze Agmo activity and gene expression in several mouse tissues. This was performed by a sensitive Agmo activity assay and quantitative PCR, respectively, in male and female knockout mice and comparing them to heterozygous and wild-type littermates.

Taken together, we could generate an Agmo knockout mouse model and will present our first results on this novel and unprecedented tool to study Agmo physiology *in vivo*.

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Immune activation, anemia, iron deficiency and outcome in patients with cardiovascular diseases

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Immune activation, anemia and a disturbed iron metabolism are frequently found in patients with cardiovascular disease and related to disease severity and outcome in these patients. The aim of this study was to investigate the association between immune activation and disturbed iron metabolism as well as their impact

on disease severity and outcome. We retrospectively analyzed data of 3316 patients from the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. Patients were followed for 10 years and parameters of iron metabolism and immune activation were correlated with each other as well as with disease severity and outcome. Neopterin and C-reactive protein (CRP) concentrations were significantly higher in patients with anemia and/or iron deficiency (ID) and rose with higher NYHA class. While CRP concentrations were significantly higher in patients with functional ID compared to absolute ID, neopterin concentrations did not differ. Circulating iron and hemoglobin concentrations as well as the transferrin saturation were negatively correlated with neopterin and CRP concentrations, while high hepcidin and ferritin concentrations were only related to higher CRP concentrations. As already shown in former studies, elevated neopterin and CRP concentration as well as anemia and ID were associated with a higher risk for death from cardio-cerebrovascular disease. Of interest, neopterin concentrations were the strongest outcome predictor, when comparing with CRP concentrations, anemia or ID. However, neopterin was only predictive for further events in patients with preserved kidney function. Since neopterin is excreted by the kidney calculation for the neopterin/eGFR ratio seems to be appropriate. Th1 immune activation, reflected by elevated neopterin concentrations, is associated with especially low circulating iron concentrations and involved in cardiovascular disease progression. Neopterin/eGFR ratio seems to be a better outcome predictor than other inflammatory markers (such as CRP), anemia or iron deficiency.

Knock on effect of periodontitis to the pathogenesis of Alzheimer's disease

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Alzheimer's disease has chronic inflammatory components which can be enhanced by systemic inflammation. There is growing evidence that chronic periodontitis drives systemic inflammation and finally

Alzheimer's disease [1,2]. Therefore, a link might exist between oral pathogens and Alzheimer's disease. This may be of special significance as there is an age-related incidence of chronic periodontitis. In this study, 20 consecutive patients with probable Alzheimer's disease were investigated [3]. Diagnosis was established by cognitive tests, routine laboratory tests and cerebral magnetic resonance tomography. In 35% of these patients with cognitive decline pathogenic periodontal bacteria were found. The presence of *Porphyromonas gingivalis*, the key pathogen and one of the species involved in chronic periodontitis, was found to be associated with lower mini mental state examination scores ($p < 0.05$) and with a tendency to lower scores in the clock drawing test ($p = 0.056$). Further, association between lower serum neopterin concentrations and the presence of *Treponema denticola* ($p < 0.01$) as well as lower serum kynurenine concentrations were found in AD patients positive vs. negative for *Tannerella forsythia* ($p < 0.05$). Data indicates a correlation of specific periodontal pathogens with cognitive decline. *T. denticola* and *T. forsythia* may down-regulate the host response in AD leading to a reduced inflammation. Albeit still preliminary, findings of our study suggest that an altered salivary microbiome may provide a causal link between chronic periodontitis and cognitive decline in AD.

To conclude, in the absence of longitudinal data our preliminary findings can only provide correlational evidence that chronic periodontal bacterial infections may be additive in the pathogenesis of cognitive decline and AD. Future longitudinal studies including periodontal disease in larger numbers of demented patients as well as age related non-demented individuals respecting the ApoE status are necessary to elucidate the specific role of the oral microbiome in neuroinflammation and neurodegeneration and its potential to prevent or delay the onset of AD. Additional longitudinal studies also should investigate the effects of certain bacterial strain consumption to improve or prevent periodontal disease in the aging population. Small molecule inhibitors to reduce the bacterial load of certain bacteria like *P. gingivalis* will be developed in the near future [4]. Thus, encompassing investigation of the symbiotic intestinal, oral as well as nasal microbiome might give additional information to define new pathways for evolutionary AD treatment.

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Bottom-up contribution in the pathogenesis of Alzheimer's dementia?

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Dementia is an increasing health problem in older aged populations worldwide. Age related changes in the brain can be observed decades before the first symptoms of cognitive decline. Cognitive impairment has chronic inflammatory components which can be enhanced by systemic immune activation resulting in inflammation or *vice versa*. Signs of an activated immune system like the increase of serum concentrations of soluble biomarkers such as neopterin or accelerated tryptophan breakdown by indoleamine 2,3-dioxygenase-1 (IDO) develop in a significant proportion of patients with dementias and correlate with the course of the disease and are also of predictive value. The changes of biomarkers concentrations were observed to be related to systemic infectious pathogens like cytomegalovirus (CMV) or bacterial content in saliva. More recently a role of the microbiome composition in AD pathogenesis has been raised. Such considerations imply that not only in the brain pathology is important for the pathogenesis of AD [1].

Interestingly enough in patients with, e.g., Alzheimer's dementia (AD) the signs of an activated immune system were noted to be more drastically expressed in the circulation than in the cerebrospinal fluid (CSF). Data implied an outside brain contribution in the pathogenesis of AD. It is still unclear how important any additional role of outside brain mechanisms can be, thereby the composition of the gut microbiome could play a prominent, probably yet underestimated, role [2].

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Immunosuppressive activity of tetrahydrobiopterin is associated with mobilization of mast and regulatory T-cells

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The natural occurring antioxidant and vitamin-like compound tetrahydrobiopterin has been shown to exert immunomodulatory activities in ischemia reperfusion injury as well as acute graft rejection models in mice. While nitric oxide synthases are involved in the attenuation of ischemia reperfusion injury, the underlying mechanisms in preventing acute rejection are still unknown. Herein, we wanted to further dissect the immunosuppressive property of tetrahydrobiopterin. A fully MHC mismatched (C3H/He to C57BL/6) mouse heterotopic heart transplantation model was used. Recipients were treated with either tetrahydrobiopterin (50mg/kg b.w.) three times a day or cyclosporine A (15mg/kg b.w.) every 24h, for six days. Syngeneic transplants and untreated allograft recipients served as controls. Six days post transplantation the graft function was assessed by palpation and inspection and the organ was finally retrieved. The severity of acute rejection was assessed by histopathological analysis according to the International Society for Heart and Lung Transplantation score, and splenocytes were analyzed by flow cytometry. The median graft functioning score at day six was significantly higher in tetrahydrobiopterin treated compared to untreated animals ($p < 0.01$) and was comparable with cyclosporine A treated ($p = ns$) as well as with syngeneic animals ($p = ns$). Histopathological analyses showed consistently severe rejection in untreated allografts and mild rejection in cyclosporine A treated as well as syngeneic grafts ($p < 0.01$ and $p < 0.03$, respectively). Tetrahydrobiopterin treated grafts showed both mild as well as severe lymphocytic infiltrates ($p = ns$). In the spleen of control, cyclosporine A treated and tetrahydrobiopterin treated animals, dendritic cells and NK cells were characterized by comparable frequencies ($p = ns$). Interestingly, tetrahydrobiopterin treated animals showed also a substantial increase

in cytotoxic T cells in the spleen ($p < 0.05$), however, displaying lower CD28 expression ($p < 0.05$). Of note, mast cells and regulatory T cells were significantly increased in tetrahydrobiopterin treated animals compared to control and cyclosporine A treated animals ($p < 0.05$). Tetrahydrobiopterin treatment leads to an increased mobilization of mast cells and regulatory T-cells. These two cells are known to create a functional tolerogenic unit and might account for the immunosuppressive property of tetrahydrobiopterin in this acute rejection model.

Personalized cardiology - an update on immune-inflammatory markers

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Atherosclerosis leading to myocardial infarction and stroke remains the main cause for mortality worldwide. Vulnerable atherosclerotic (AS) plaques are responsible for these life-threatening clinical endpoints. Atherosclerosis is a chronic, complex inflammatory disease with interactions between metabolic dysfunctions, lipids, vascular, and immune cells. Although, macrophages and T cells have been in the focus of research since the last ten years, B cells producing antibodies and regulating T and natural killer (NKT) cell activation are more important than former thought. New results showed that the B cells exert a prominent role with atherogenic and protective facets mediated by distinct B cell subsets and different immunoglobulin effects. These new insights come amongst others from observations of the effects of innovative B cell targeted therapies in autoimmune diseases like systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). These diseases associate with AS, and beneficial side effects of B cell subset depleting (modifying) therapies on atherosclerotic concomitant disease has been observed. Moreover, the CANTOS study (NCT01327846) showed impressing results of immune-mediated inflammation as a new promising point of action for the eradication of fatal atherosclerotic endpoints.

Neopterin as biomarker in patients treated with immunotherapy

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Biomarkers represent an integral part of management strategy in medical oncology. Historically, most attention has been focused on biomarkers associated with the tumors cells. With the advent of immunotherapy that has transformed the therapeutic approach across a spectrum of neoplastic disorders, a need emerged for biomarkers that would predict the response to treatment. Neopterin as a biomarker of cellular immune response is increased across a spectrum of different disorders associated with immune activation, including malignant tumors. It has been previously demonstrated that serum and urinary neopterin concentrations are increased by anticancer treatment, including systemic administration of cytotoxic drugs or irradiation. Increased production of neopterin has also been demonstrated after administration of cytokines, and, more recently, immune checkpoint inhibitors. Increased production of neopterin correlates with the conversion of tryptophan to kynurenine that may represent a major immunosuppressive mechanism. There are emerging data that changes of neopterin concentrations as well as kynurenine/tryptophan ratio may be used to monitor response to treatment and associated toxicity.

Tryptophan metabolism, stress and feather pecking in domestic chickens.

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Tryptophan (TRP) metabolism in mammals can be influenced by stress, leading to an increase of TRP breakdown towards the kynurenine (KYN) pathway. In young laying hens, however, the TRP metabolism along the KYN axis may differ and respond differently to stress.

As a part of a larger project, we investigated the effect of Unpredictable, Repeated Stressors (URS) on plasma concentrations of TRP and KYN in pullets housed in enriched floor pens. White Leghorn pullets (n=354, 10 weeks of age) came from 3 different lines of birds bred for their high, low, or unselected feather pecking phenotype (120±2 birds/line). These birds were systematically assigned to 6 stressed (S) or 6 non-stressed pens (NS) (10 birds/line/pen).

The 6 S groups were exposed to social and non-social URS for 3 weeks while the NS groups were left undisturbed.

Blood samples were collected one-hour post-feeding before and after the 3 weeks of URS, and analyzed using high-performance liquid chromatography. A generalized linear mixed model was used to assess the effect of the genetic lines and URS on plasma concentrations of TRP, KYN as well as their ratio, calculated as an indication of the activity of TRP catabolism. The birds were considered as the experimental unit.

Our results revealed that birds from the line bred for its low FP phenotype showed significantly higher levels of plasma TRP ($P < 0.001$) compared to birds bred for their high FP phenotype. TRP (+6%, $P < 0.001$), KYN (+33%, $P < 0.001$) and their ratio (+26%, $P < 0.001$) significantly increased over the URS period in all birds. The URS treatment did not significantly impact plasma TRP, KYN or their ratio ($P > 0.05$).

These preliminary data show that differences in TRP metabolism already exist in young chickens bred for extreme FP phenotypes. Further research will also investigate the immune status of the birds and its role in TRP metabolism.

Rapid cure of a chronic wound infected with multi-resistant *Staphylococcus aureus* in an elderly patient with N-chlorotaurine and N-bromotaurine. A Case Report.

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N-chlorotaurine (NCT) and N-bromotaurine (NBrT) are endogenous anti-infective active halogen compounds with additional anti-inflammatory properties. Here we present a case report with successful topical application of a combination of both. A 91-year-old female with a high-stage renal disease, diabetes type 2, and considerable weakness acquired a wound from a bush scratch on the right lower leg. The wound became infected and was treated unsuccessfully with topical aminoglycosides and iodine. It was purulent and measured 7 x 5 cm when the patient entered the hospital. Treatment with systemic antibiotics including vancomycin was not effective, too. Cultures revealed a methicillin-resistant *Staphylococcus aureus*. Therefore, a change to antiseptic treatment was decided. Topical application of 1% NCT spray and 1% NBrT spray was started every 30 min with an interval of 5 min between each agent. This regimen led to rapid disappearance of the purulent exudate within one day, and rapid epithelialization and rapid healing within 3 days. To avoid relapse, the application was continued four times daily for a further four days. No complications or adverse effects except for slight itching for a few minutes after application occurred. NCT and NBrT can be applied also in combination in infected wounds. Microbicidal and anti-inflammatory effects found *in vitro* are transferable to the *in vivo* situation. The single effect of NBrT and related bromamines remains to be investigated.

Lifestyle of patients with bipolar disorder and its relationship to the tryptophan-kynurenine metabolism.

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The effect of psychoeducation in bipolar disorder (BD) has already been validated. Programs (e.g. by Wagner & Bräunig) impart knowledge about disease-specific facts, lifestyle-interventions and medication for BD patients. They attempt to improve the understanding of the disease and try to extend knowledge about physical activity (PA), healthy diet, time management, stress regulation, and recognition of early symptoms. Especially for PA, positive effects have been reported for BD patients. Nevertheless, there is a lack of knowledge about the underlying metabolic pathways that are involved in this relationship. Tryptophan-kynurenine pathways show abnormalities in BD patients and can be linked to acute manic and depressive phases as well as to inflammatory processes. The relationship between PA and tryptophan-kynurenine metabolism in BD has – to our knowledge – not been explored yet. To investigate the effects of PA on tryptophan-kynurenine pathways in BD, we asked 51 BD patients and 49 healthy controls about their PA. Furthermore, we measured several medical and psychological parameters (serum levels of tryptophan metabolites, cognitive testing, self-rating questionnaires, and medical ratings). After analysis with ANCOVA, we found significant interactions between the variables (PA, sex and group). Female BD patients showed high kynurenine (and quinolinic) levels for no PA and much PA as well as low kynurenine (and quinolinic) levels for moderate PA. In males and healthy controls, kynurenine (and quinolinic) levels were higher only for moderate PA, comparable to recent literature about inflammatory processes due to PA. Regarding this results, future psychoeducation programs should consider this information about the possible implications of PA on inflammation-regulation for their recommendations. Nevertheless, further studies are needed to evaluate the sex-specific positive effects of moderate PA for BD patients.

Sex differences in zonulin in affective disorders and associations with current mood symptoms

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The bidirectional connection between the brain and the gut within psychiatric entities has gained increasing scientific attention over the last years. As a regulator of intestinal permeability, zonulin acts as a key player on the interface of this interplay. Like several psychiatric disorders, intestinal permeability was associated with inflammation in previous findings. In this study we explored differences in zonulin serum levels in currently depressed versus currently euthymic individuals with an affective disorder. We formed groups using a cut-off score of 10 on the Hamilton Depression Scale. Further, we explored sex differences and possible influences on zonulin and affective symptoms like medication, age, body mass index, and smoking status. Serum zonulin was significantly higher in females independent from affective status and in the euthymic subgroup but not in depressed subjects. There was no difference in zonulin serum levels between individuals taking antipsychotics and antidepressants and those without. We found no correlation between zonulin serum levels and depression severity. Increased serum zonulin levels as a proxy for increased intestinal permeability in euthymic women may indicate a state of elevated susceptibility for depression-inducing stimuli.

Metabolic regulation of tumor progression: the role of tryptophan catabolism

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Metabolic control of anti-tumor immune responses is recognized as an important mechanism contributing to cancer progression. Catabolism of tryptophan along the kynurenine pathway represents one such mechanism. The expression and activity of the tryptophan-catabolizing enzymes indoleamine 2,3-dioxygenase 1 (IDO1) or tryptophan-2,3-dioxygenase (TDO) are upregulated in cancers and/or in the tumor microenvironment. These findings sparked clinical trials investigating small molecule inhibitors of IDO1 and TDO2 for cancer immunotherapy. However, a phase III clinical trial of the IDO1 inhibitor epacadostat in combination with anti-PD1 antibodies failed in advanced melanoma. This talk will give an overview of tryptophan catabolism in cancer, its regulation, the molecular mechanisms underlying its effects, methods to measure tryptophan metabolites and the challenges in developing drugs that interfere with this pathway.

Effect of minocycline on tryptophan metabolism in human PBMC

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Minocycline hydrochloride is a broad-spectrum long-acting antibiotic, which can cross biological barriers due to its lipophilicity. The compound promotes neurogenesis and neuroplasticity, reduces oxidative stress and glutamate excitotoxicity and attenuates decrease of serotonin, dopamine, norepinephrine and their derivatives. By acting on a wide variety of immune cells and mediators, minocycline is able to reduce inflammation and may exert the anxiolytic effects through modulating neuroinflammation. In the course of the immune response, enzyme indoleamine 2,3-dioxygenase (IDO-1) is activated by interferon- γ (IFN- γ) in human macrophages and dendritic cells. Activation of the IDO enzyme and the resulting decrease in plasma tryptophan levels appears to be a crucial link in the relationship between cytokines and depression. The aim of our study was to monitor a potential interference of minocycline with tryptophan catabolism in human peripheral mononuclear

cells (PBMC) that were activated or not with the mitogen phytohemagglutinin (PHA). Data show that minocycline treatment suppressed PHA-stimulated tryptophan catabolism at pharmacologically relevant concentrations.

Introduction to the physical, chemical and biological environment in relationship to neuropsychiatric health

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In behavioral sciences the term *environment* is often limited to the psychosocial one, many times equated with socioeconomic adversity, with abuse, neglect or traumatic events in general. As environmental factors that primordially interact with our time-keeping, hormonal, immune and central nervous system- we emphasize the need to study that natural environmental factors that can influence brain and behavior evolutionarily, contemporaneously, and developmentally. These include physical factors (such as light, temperature, air pressure, but also physical trauma), chemical- (such as chemical composition of food water, air) and biological (such as allergens and microbes). The *evolutionary environment* is the natural environment that selected our ancestors, during “*evolutionary times*.” Given that we, in particular in the Western world, have continuously isolated ourselves in a microclimate with controlled light and temperature and reducing, but not minimizing, the exposure to outdoor aeroallergens, and pollution. Through selection, it is likely that in certain cases our neurocircuits, neuroendocrine connections, and overall regulatory processes are more adapted to the environment of our ancestors rather than to our contemporaneous environment. For example, physiological and clinical changes in seasonal affective disorder (SAD), winter type, resembling the biological and behavioral changes in photoperiodic animals, may have adaptive function in an environment characterized by a bottleneck between reduced caloric resources meeting increased metabolic demands. Yet, the same changes, contemporaneously, may represent liabilities from a functional (occupational, academic, athletic) health

and social standpoint (e.g. fatigability and sleepiness in SAD patients in the winter, their decreased interest in social interactions, increased appetite, food intake and BMI). Examples of environmental interventions based on the specific deprivations in the environment include bright light exposure for SAD [1] and for circadian rhythm disorders [2, 3], Vitamin D treatment of immune dysregulation secondary to Vitamin D deficiency [4], and soil saprophytic bacteria administration in conditions that could be, in part, a consequence of the overly hygienic environment we live in [5] including allergies, potentially anxiety disorders and suicidal behavior [6].

Seldom is one therapeutic element of a specific environment as effective as the fuller environmental exposure, for instance, the light of summer is not as effective as summer in improving mood in SAD patients [7]. Allergic rhinitis and asthma [8] often translate the effect of airborne allergens on mental health, not uncommonly via disturbances of sleep [9]. The nasal cavity [10] represents the point of entrance of many factors that induce and exacerbate inflammation in the CNS- such as pathogens, including respiratory viruses with neurotropic potential (such as coronaviruses and influenza viruses) [11] and aeroallergens [6]. Further pointing towards nasal cavities, smell identification changes, unilaterally, have been associated with seasonality of mood [12]. Other environmental factors that induce mental health changes involve a GI pathway- such as in *Toxoplasma gondii* infection (linked with depression and suicidal behavior), and gluten exposure in individuals positive for gliadin antibodies [13].

Convergent approaches ranging from macroepidemiology [14], pharmacoeology [15], postmortem [16], and animal research [17], with a necessary focus on women [18] and minorities [19], as well as interactions between two or more potentially synergistic or antagonistic elements of environmental influences that are particularly important [20] could separate “knowledge” from “findings”. Nonlinear dynamic approaches to weather – mood interactions [21], as well as, increasingly used, machine learning techniques and alternative nonlinear modeling of environment-genes interactions [22, 23] may bring us closer to linked phenomena as compared to simple linear models. Intersections between genetics of conditions triggered by environmental changes (such as SAD) and of mental illness such as schizophrenia [24], studying behavioral effects of combined adverse psychological (such as induced helplessness of medical diagnoses, when persistent or chronic) and biological factors (such as specific allergen sensitization and exposure [25]), investigating the

microbiome of the built environment [5, 26], comparing in parallel design the effects of psychological and environmental based interventions [27], and combined specific triggers - vulnerability dipoles in predicting worsening of symptomatic functional scores [28] are expected to result in major advances in environmental mental health studies. Conversely, studying both the link of mental illness and infections, but also of anti-infective agents – potentially through their destructive effect on microbiota [29] and thus impairing their capability to “socialize” the immune system and prevent overly too intense, too prolonged and too unremitting immune activation after infectious, mechanical and psychological triggers, is likely to result in novel treatment goals and means. A focus on well controlled short term interventions, in particular identifying if there are rapid effects after very brief interventions could affect relief and result in more positive expectations of treatment, could further increase the potential for better adherence and remission with environmentally based interventions such as light treatment and represent prognostic indicators of the end of study outcome [30] providing early symptomatic relief [31].

Studying environmental challenges in special populations with known alterations in the immune system, such as pregnant women [32], and in severely symptomatic individuals, such as the highly impulsive – aggressive patients with Intermittent Explosive Disorder [for investigating aggression as an intermediate phenotype of suicidal behavior [33] could further differentiate subpopulations by their sensitivity to specific environmental factors that are ideal, acceptable, minimally or severely adverse. Ultimately, these efforts, in the long run, will likely improve our capability to better understand, prevent and respond to increasing challenges responding induced by climate change.

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Dental health and sleep-wake problems in the Old Order Amish

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Growing evidence connects periodontal disease, a major and modifiable cause of local systemic inflammation, with metabolic and cardiovascular morbidity and mortality, and mental illness. Sleep has been also predictively associated with metabolic and mental morbidity and mortality, and recently linked with periodontal disease. We are now evaluating associations between self-reported insomnia measures and surrogate indicators of periodontal disease

in a population with a very low prevalence of smoking, a major confounder in previous studies.

Sleep and dental questionnaires based on PSQI were obtained from 3881 Old Order Amish from Lancaster county. Difficulty falling or staying asleep, sleep quality and problems during the day due to poor sleep, were related to self-reported loose teeth, false teeth and partial false teeth using linear models with adjustment for age and sex.

Significant positive associations emerged between problems falling asleep and loose teeth ($p = 0.05$), problems staying asleep and false teeth ($p < 0.05$), sleep quality with loose and false teeth ($p < 0.05$). Problems during daytime due to poor sleep were associated with loose ($p < 0.05$) and false teeth (for any false teeth $p < 0.003$, for full false teeth $p < 0.0001$ - the only associations resisting Bonferroni correction).

Limitations include not accounting for family aggregation, limited generalizability, and not fully differentiating between respiratory vs. non-respiratory sleep impairment, and periodontal vs. traumatic dental pathology. Yet the results of our study, that minimizes the strong potential confounding by smoking, confirm associations between sleep-related problems and dental illness, and justify future longitudinal and interventional research to dissect causality and identify multitarget treatment modalities.

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Homocysteine and cognitive function in bipolar disorder

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There is evidence that homocysteine might be involved in the biological underpinnings of psychiatric disorders as it interacts with NMDA receptors, initiates oxidative stress, induces apoptosis, triggers mitochondrial dysfunction and leads to vascular damage. In this context, homocysteine is known to serve as an atherosclerotic factor. This of special interest as it is known that individuals with psychiatric disorders in a high proportion suffer from somatic disorders which lead to decreased quality of life and significantly lower life expectancy compared to mentally healthy individuals. Elevated homocysteine levels might also contribute to cognitive impairment that is widely observed among individuals with affective disorders and schizophrenia.

In this study 216 individuals with bipolar disorder and 119 healthy controls were included and differences between the two groups analyzed. Furthermore, associations with clinical parameters were calculated.

Homocysteine levels were significantly higher in individuals with bipolar disorders compared to healthy controls. Nevertheless, if controlled for relevant covariates (age, sex, body mass index and smoking) no significant difference between the groups could be found. Men had significantly higher homocysteine levels compared to women in patients as well as controls. Furthermore, age correlated significantly with homocysteine in bipolar men as well as healthy women.

Interestingly, we found no significant correlation between homocysteine and cognitive function in individuals with bipolar disorder, while in healthy controls lower verbal memory was associated with higher homocysteine levels.

Elevated serum homocysteine levels have been observed in some studies with patients with BD. Reports suggest that sex and age are important covariates insofar that older male patients are the most vulnerable cohort; which was also present in our study. Missing correlations with cognition in individuals with bipolar disorder might be due to further covarying variables (as medication or illness specific symptoms) or due to overlapping biochemical processes. Furthermore, different subsamples of individuals, e.g. these with high or low inflammatory status, should be investigated.

The role of the neuro-immune axis in high trait anxiety: modulatory effects of minocycline

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High-trait anxiety is a substantial risk factor for developing anxiety disorders and depression. While inflammation has been identified to contribute to stress-induced anxiety, less is known about potential dysregulation in the inflammatory system of high-trait anxiety individuals. In a mouse model of high trait-anxiety (HAB), we revealed enhanced density and average cell area of microglial cells (using Iba1 as a marker) as well as increased phagocytic (CD68+Iba1+) activity of the microglia in the dentate gyrus (DG) compared to normal trait-anxiety (NAB) controls. Besides aberrant neuro-inflammation, we also observed higher levels of pro-inflammatory plasma cytokine levels (eg. IL-2 and IL-23) in HABs. Minocycline was used to assess the capacity of a putative microglia ‘inhibitor’ in modulating hyperanxiety behavior in HABs. Chronic oral minocycline indeed reduced HAB hyperanxiety, which was associated with normalization of dysregulated plasma cytokines and Iba1+ and CD68+Iba1+ cell densities in the DG. Addressing causality, long-term microinfusions of minocycline directly into the DG of HABs was sufficient to reduce hyperanxiety-related behavior, indicating that neuroinflammation in the DG at least partially contributes to the maintenance of pathological anxiety. Taken together, our results support the role of inflammation in high trait anxiety and its risk for adulthood pathologies. Furthermore, a pro-inflammatory bias may be a mechanism contributing to the increased risk of predisposition towards high trait-anxiety. Drugs possessing anti-inflammatory properties, such as minocycline, thus may be beneficial as

a treatment option in patient sub-groups displaying high trait anxiety and aberrant inflammatory markers.

Alkylglycerol monooxygenase in human adipocyte stem cell differentiation

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Alkylglycerol monooxygenase (AGMO) is involved in ether lipid metabolism by breaking down alkylglycerols and lyso-alkylglycero-phospholipids in a tetrahydrobiopterin dependent manner [1]. We know from our experiments that AGMO is expressed in several murine tissues including adipose tissue and the 3T3-L1 preadipocyte cell line. However, data on the role of AGMO and ether lipids in human adipocyte physiology and metabolism is very limited.

To address a potential role for AGMO and its metabolites in human adipocyte physiology and differentiation processes we used tissue samples of patients that underwent abdominoplasty surgery and studied the enzymatic activity of AGMO in *in vivo* differentiated adipocytes and during the course of *in vitro* adipose conversion of the human stromal vascular fraction isolated from subcutaneous adipose tissue of different donors. Tetrahydrobiopterin levels were determined in whole tissue samples in parallel. Additionally we looked at ether lipid metabolites by supplementation of human adipose derived stem cells with a fluorescently labelled ether lipid during the course of *in vitro* adipocyte differentiation.

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Dynamic psycho-immunological interdependencies of stress experience in a psychodynamic interview

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While there is much evidence for the beneficial long-term effects of psychodynamic psychotherapy regarding stress related disorders, little is known about the immediate immunological effects on the stress system during in-depth conversations between client and therapist. Building on the growing evidence, that daily psychosocial stressors interfere with immunological processes and therefore disease, this study aims to uncover the poorly understood interplay between psychological and immune parameters during an 80 min psychodynamic psychotherapy session. To do so, we used combined measures (qualitative and quantitative) to simultaneously investigate a client's and a therapist's immune parameters as well as psychodynamically relevant content of their conversation. For this purpose, in both, an experienced psychotherapist and a client suffering from chronic autoimmune disease (systemic lupus erythematosus; age 29 years), blood samples were continuously drawn in 2.5 min intervals for consecutive measurement of stress system parameters (33 equidistant measurements) while audiovideotaping their conversation. Stress system parameters (e.g. neopterin, kynurenine/tryptophan, phenylalanine/tyrosine, nitrite) were measured through blood samples while both the client's and the therapists verbal and non-verbal stress experience was coded via conversation analysis and consensus rating through trained coders. From these measurements several psychological and immunological time series were constructed. Findings from time-series analysis (e.g. ARIMA modeling, cross-correlation analysis) on the complex psycho-immunological interrelationships in the client and therapist under study will be presented. This single-case study aims to reveal new insights of psychotherapeutic effects on the immune system, narrowing the gap between

biological, psychological and social models of stress and disease to promote awareness that psychotherapy can bring about direct and cost-effective improvements in the healthcare sector.

From early to late pregnancy, tryptophan plasma levels decrease in *Porphyromonas gingivalis* IgG K antigen-positive but not in K antigen-negative women

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Porphyromonas gingivalis (*P. gingivalis*) is the keystone periodontal pathogen causing chronic periodontitis. During pregnancy, *P. gingivalis* can translocate to the placenta and is associated with hypertension, insulin resistance and adverse obstetrical outcomes, such as pre-term birth. One of several virulence factors of *P. gingivalis* is its capsule. Antibodies to the capsular K1-7 antigens have been used to differentiate *P. gingivalis* serotypes according to the capsular polysaccharide. In pregnancy, an upregulation of inflammation occurs towards the end of pregnancy and has been incriminated in peripartum

depression. Inflammation and stress have been shown to decrease levels of tryptophan and increase kynurenine, via enzymatic activities of IDO and TDO enzymes upregulated by pro-inflammatory cytokines and glucocorticoids, respectively in several different studies. As tryptophan is a precursor of serotonin, a neurotransmitter centrally involved in affective and behavioral regulation, its reduced level could result in new onset or exacerbation of psychiatric symptoms, syndromes, and behavioral abnormalities perinatally. Elevation of kynurenine may lead to the formation of NMDA agonists and antagonists that could induce mood and behavioral dysregulation and cognitive deficits.

Fifty-eight pregnant Hispanic women (age 33 ± 5 years) in central Florida were followed as part of a broader study on infection and pregnancy outcomes, and blood was obtained at three time-points (early, mid and late pregnancy). Levels of tryptophan and kynurenine were measured by high pressure liquid chromatography (HPLC). *P. gingivalis* IgG antibody titers to capsular serotypes K 1-7 were measured by ELISA. Capsular antibody positivity (K-positivity) was defined as the top 25 % percentile (top quartile) separately for all 7 *P. gingivalis* K-capsular antigens. If positivity was found to any serotype- then the woman was defined as “K positive.” If the plasma sample was negative to all the capsular antigens, then the woman was defined as K antigen “negative.” Statistical analysis consisted of repeated-measures ANOVAs stratified by *P. gingivalis* seropositivity at the first visit (V1). Tryptophan and kynurenine and their ratio were dependent variables.

In late pregnancy, tryptophan was significantly lower (ANOVA repeated measures $p = 0.007$) relative to mid (post-hoc $p < 0.005$, only finding resisting adjustment for multiple comparisons) and early pregnancy (0.014), in women who were K antigen seropositive. In K seronegative women there were no significant differences. There were no statistically significant differences in kynurenine or kynurenine/ tryptophan ratio between any time points.

Our results suggest that seropositivity to *P. gingivalis* K serotypes may augment and/or interact with the proinflammatory environment of late pregnancy leading to low tryptophan and subsequent reduced serotonin synthesis. This may contribute to an increased perinatal psychological vulnerability to depression, anxiety, mood instability, and behavioral dysregulation including suicide attempts.

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USA. The findings and conclusions in this study are those of the authors and do not necessarily represent the official positions of the Veterans Health Administration or the National Institutes of Health.

Oral tryptophan supplementation in humans and urinary excretion of kynurenine and neopterin

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This study investigated the influence of oral tryptophan application on the urinary excretion of tryptophan and kynurenine and the related product neopterin. Six volunteers (three males and three females, aged between 23 and 26 and one with 78 years) ingested 1.5 g tryptophan orally (3 x 500 mg capsules) after they gave informed consent. Urine collection was performed in the following way: On Saturday evening the urine was collected, thereafter 500 mg tryptophan was ingested. Sunday morning another two capsules containing 500 mg tryptophan each were ingested, and on Sunday evening the urine was collected again. The urine was brought to the laboratory at room temperature and kept at -20°C and analyzed using high performance liquid chromatography [1].

After the tryptophan load, the concentrations of tryptophan and kynurenine increased significantly in the urine ($p = 0.03$), showing that the absorption of the amino acid via the gastrointestinal system was intact. Concentrations of neopterin and creatinine as well as the neopterin to creatinine and kynurenine to tryptophan concentrations were not influenced by the oral intake of tryptophan.

The increase of kynurenine concentrations observed upon tryptophan supplementation results most likely from an enhanced activity of hepatic tryptophan 2,3-dioxygenase (TDO). We conclude that oral application of tryptophan leads to an increase of tryptophan in urine

and most probably also blood levels increased. Unlike TDO, indoleamine 2,3-dioxygenase (IDO), is upregulated by immune stimuli but is not directly influenced by blood tryptophan concentrations. In addition, the concomitantly measured concentrations of the immune system biomarker neopterin and neopterin to creatinine did not change at all, supporting the conclusion that no influence on immunity was induced by tryptophan loading. No obvious influence of oral tryptophan on the clinical presentation of the volunteers was noticed after this single application of tryptophan.

Tryptophan supplementation is discussed as strategy for increasing tryptophan supply to the brain. Frequent supplementation may however influence on down-stream products like quinolinic acid and kynurenic acid which is not addressed in this preliminary study. Thus, further aspects of safety such as increasing kynurenine levels or other downstream products still have to be explored.

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From hidden fever to silent inflammation - inflammation and anti-inflammatory concepts from Tibetan Medicine

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In Tibetan Medicine health is seen as a dynamic and personal equilibrium of three principles *Lung*, *Tripa* and *Beken*. *Tripa* is the hot principle responsible e.g. for body temperature, catabolic processes, and digestion. In excess it causes so called hot diseases like different kinds of 'fever'. 'Hidden fever' is a multi-layered condition where *Tripa* is combined with cold *Lung*- and/or *Beken*-disturbances. It therefore at first presents as a 'cold' disorder despite being of an underlying hot nature. The hidden aspect of fever or inflammation is a known phenomenon also in Western medicine, where it is called silent inflammation, a chronic low-level inflammatory state with the cardinal signs of inflammation missing. Silent inflammation has been causally connected to different diseases such as vascular aging and arterial stiffness, atherosclerosis, diabetes mellitus, Alzheimer's disease, and certain forms of cancer. It is in turn promoted by such inflammatory diseases and characteristically increases with advancing age, a process called inflammaging. Tibetan Medicine

has developed specific remedies to treat ‘hidden fever’, among these the complex herbal formula *Gabur 25* (camphor 25), which is available as medicinal product in different European Countries (in Austria under the name PADMA CIRCOSAN, Zul.Nr.: HERB-00037). From the point of view of Tibetan Medicine this formula primarily reduces the *Tripa* principle and therefore heat in the blood vessels (‘blood fever’). Furthermore, it reduces the stagnatory *Beken* principle and increases the mobile and moving *Lung* principle. In the Western context this formula has been used as approved medicine since over 40 years against circulatory disorders (PADMA 28 in Switzerland). Over 30 clinical trials have found an efficacy in several chronic inflammatory diseases, with the highest level of evidence in peripheral arterial occlusive disease (PAOD, Fontaine stage II) [1, 2]. Numerous experimental studies found a whole range of mechanisms of actions, that are related to the development of atherosclerosis and diabetic complications [3]. Among these are several direct and indirect anti-inflammatory, anti-oxidative and cell protective mechanisms, e.g. reduction of pro-inflammatory cytokines IL-1 β , IL-6, TNF- α [4] as well as an inhibition of neopterin production and tryptophan degradation [5]. The scientific data on PADMA CIRCOSAN/PADMA 28 confirms the traditional use of the formula in Tibetan Medicine. The comparability of ‘hidden fever’ with the modern notion of silent inflammation demonstrates, how ancient concepts may be interpreted and of relevance in modern medicine.

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Another novel gene in ether-lipid metabolism

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Ether lipids are abundant glycerol-based lipids and phospholipids which are characterized by attachment of a fatty alcohol by an ether bond to the *sn*1 position of glycerol. This feature distinguishes them from the more commonly studied ester lipids which have a fatty acid attached by an ester bond at this position. The initial three steps of the biosynthesis of ether lipids in animals are carried out in peroxisomes by *GNPAT*, *AGPS* and *DHRS7B* genes. Subsequent steps of the biosynthesis occur in the endoplasmic reticulum, and the genes required for these steps are less well characterized. A subclass of ether lipids, the plasmalogens, contain a characteristic double bond adjacent to the ether linkage at *sn*1, resulting in the formation of a vinyl ether. This characteristic double bond is introduced by the enzyme plasmalogen desaturase (E.C. 1.14.99.19).

In 2010 we succeeded in assigning a gene to the ether lipid-degrading, tetrahydrobiopterin-dependent enzyme alkylglycerol monooxygenase [1]. Stimulated by this achievement, we aimed at characterizing also the gene for plasmalogen desaturase. We first set up an assay to quantify the enzymatic activity [2], and then selected candidate genes by bioinformatic selection criteria which included protein motifs and correlation of enzymatic activity with RNA expression data in human cell lines and in mouse tissues. This led to the identification of *TMEM189* as gene encoding plasmalogen desaturase [3]. Working on the characterization of a bacterial light response, another group reached the same conclusion by a completely different approach [4].

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Winter and summer patterns of seasonality are associated with specific environmental sensitivities in the Old Order Amish

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Among the many potential threats faced by the modern world, global climate change has received widespread attention. The anticipations include changes in temperature, increasing frequency of heat waves, increases in pollen counts and increased severity of viral epidemics and pandemics. These changes are expected to have substantial impact on respiratory, cardiovascular and neuropsychiatric mortality and morbidity. Seasonal changes in mood and behavior (seasonality) and seasonal affective disorder (SAD), characterized by seasonal onset of mood, neurobehavioral and metabolic symptoms [1] and spontaneous remission in other seasons, are particularly sensitive to maladaptive interactions between seasonal environmental triggers and underlying biological systems. For SAD with winter seasonal pattern (W-SAD)- the trigger is the environmental decrease in light and photoperiod, the vulnerability is physiological photoperiodism, and the corrective treatment is exposure to light [2] or SAD-targeting CBT [3]. For SAD with summer seasonal pattern (S-SAD), less studied than W-SAD, the proposed triggers include heat [4] and aeroallergen exposure [5], the vulnerabilities include thermoregulatory dysregulation and sensitization to seasonal aeroallergens

[6] and the treatment may involve exposure to cold and allergy targeting pharmacology, respectively. Therefore, we planned to explore environmental sensitivities of mood in the Old Order Amish, who spend more time outdoors and do not use power-line artificial light and air conditioning in their homes, thus being less isolated from the natural macro-environment. Previous research in the Amish has identified considerable seasonality [7], low prevalences of SAD [8], increased mood sensitivity to pollen in association with a summer pattern of seasonality [9], and a good test-retest reliability of the Seasonal Pattern Questionnaire (SPAQ) [10]. For the first time we are now reporting on the associations of self-reported mood worsening with *multiple* environmental variables in relationship to three patterns of mood changes- summer, winter and neither. We hypothesized that mood worsening with light-deprivation will be negatively associated with a winter seasonal pattern of mood and behavior, and that mood worsening with heat and high pollen will be positively associated with a summer seasonal pattern of mood and behavior.

Seasonal pattern assessment questionnaires (SPAQ) were mailed to 2260 Amish individuals who were previously enrolled in multiple studies conducted at University of Maryland, and caseness for SAD and seasonal pattern was determined according to previous publications [7]. The cases for SAD were too few for adequate statistical power; thus we focused on seasonal patterns of mood change. Logistic regression analysis was conducted with and without adjustment for age, gender, and awareness.

The prevalence of winter pattern of mood seasonality was 12.5% among men, 16.0% among women; summer pattern of mood seasonality was 13.3 % in men, 9.6% in women. The majority of participants, 74.2% men, and 75.4% reported no seasonal pattern of mood changes. After adjustment for age and gender, the winter pattern of seasonality was associated with mood worsening with cloudy days (OR = 3.089, CI: 2.165, 4.409; $p < 0.001$) and mood worsening with short days (OR= 3.642, CI: 2.616, 5.071; $p < 0.001$). After adjustment for age and gender the summer pattern of seasonality was positively associated with mood worsening on hot days (OR= 3.749, CI= 2.142, 6.564; $p < 0.001$) and with mood worsening on days with high pollen counts. (OR= 2.243, CI= 1.471, 3.420; $p < 0.001$). All these results were robust to adjustment to awareness of SAD and its treatment.

In conclusion, we have confirmed the hypotheses that winter pattern of seasonal changes in mood and behavior are linked with mood sensitivity to light deprivation, and that summer pattern of seasonal changes are linked

with mood sensitivity to heat and high pollen counts. Future studies should include larger samples or with higher prevalences of SAD, with clinical, environmental and physiological measurements and interventional paradigms. Clinically, in summer SAD it may be important to identify specific mechanisms, e.g. thermoregulatory vs. aeroallergen sensitizations and exposures, as this may lead to divergent clinical approaches. Moreover, immune changes in response to heat and allergy may interact additively, synergistically, or antagonistically. Ultimately, the research on summer SAD, heat and allergen mood sensitivity in populations that do not use air conditioning may contribute to increased knowledge on how to increase our resilience to climate change, as well as the design of and adaptation to future highly controlled microclimates (e.g. interplanetary travel).

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