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Abstracts*)

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Effects of campherol on *in vitro* models of inflammation

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Campherol (kaempferol, 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one) is a natural flavonoid found in many food and medicinal plants e.g. *Ginkgo biloba*, *Tilia spp*, *Equisetum spp.* A variety of pharmaco-relevant activities have been reported for campherol, including antioxidant, anti-inflammatory, antimicrobial, cardioprotective and estrogenic/antiestrogenic properties. Further, *in vitro* studies showed anti-proliferative activities and the induction of apoptosis in several cell lines. Herein, we investigated the impact of campherol on tryptophan breakdown and neopterin formation in human peripheral blood mononuclear cells (PBMC), and on the activation of the redox-sensitive transcription factor nuclear factor-κB (NF-κB) in myelomonocytic THP-1-blue cells. In the course of the immune response, pro-inflammatory cytokines stimulate the enzyme indoleamine 2,3-dioxygenase (IDO1) to convert the essential amino acid tryptophan into kynurenine. The kynurenine to tryptophan ratio can be used as estimate for IDO activity. In parallel, GTP-cyclohydrolase I (GCH) is induced to produce neopterin. Increased neopterin concentrations and accelerated tryptophan breakdown indicate the activation of cell-mediated immunity. The activation of NF-κB is a key mechanism in the regulation of immune activation and stress response. PBMC were stimulated with the mitogen phythaemagglutinin (PHA). Activated T-cells release pro-inflammatory cytokines, among them interferon-γ (IFN-γ), leading to an enhanced formation of neopterin and tryptophan breakdown. Additional treatment with campherol at non-toxic concentrations (6.25-100 μM) was able to reduce tryptophan breakdown, kynurenine formation and neopterin production significantly in PHA stimulated cells. All effects showed dose dependency. No similar effects could be observed in unstimulated PBMC. THP-1-blue cells are reporter cells that were stably transfected with a construct containing a NF-κB/AP1 binding site in front of a secreted embryonic alkaline phosphatase (SEAP) reporter gene. Stimulation of myelomonocytic THP-1-blue cells with lipopolysaccharide (LPS) resulted in the activation of NF-κB, which could be measured by assessing the SEAP activity in a colorimetric assay. In contrast to other published data, additional treatment with campherol showed a dose dependent increase in NF-κB activation after stimulation with LPS, while unstimulated cells were not affected. The *in vitro* data obtained from the PBMC assay could provide a biochemical background that at least partially explains the anti-inflammatory activity of campherol, due to its interference with key signaling cascades in cellular immune response. Additional experiments will be needed to investigate the effect of campherol on NF-κB activation in more detail.

Neopterin, tryptophan breakdown and tyrosine metabolism in acute alcohol withdrawal

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The mechanisms of tryptophan-kynurenine metabolism in chronic alcoholism and acute alcohol withdrawal are not completely understood. We investigated the role of immune activation in tryptophan metabolism during the first 2 weeks of alcohol abstinence in chronic alcohol-dependent patients admitted to alcohol withdrawal treatment in an in-patient unit. Alcohol dependence was assessed using Alcohol Use Disorder Identification Test (AUDIT). Detailed data upon alcohol consumption were acquired by means of Timeline Follow Back scale (TLFB). Blood samples for biochemical analysis were collected on first, fifth and 10th days of treatment. Eleven patients have been investigated so far (9 men and 2 women). Age ranged between 25 and 59 yrs. All patients were diagnosed with severe alcohol-dependence of at least several years and reported daily alcohol consumption (239 g per day in average) prior to admission. Our preliminary results show that neopterin concentrations correlate significantly with both kynurenine-tryptophan and phenylalanine-tyrosine ratios as measured by Spearman rank correlation test and Mixed Linear Model analysis over all three sampling points (first, fifth and 10th days). There is a tendency for neopterin, kynurenine-tryptophan ratio and phenylalanine-tyrosine ratio to increase between the first and 10th day of the study but this finding did not reach the level of statistical significance. Our preliminary data support the previous findings on a relationship between immune activation and tryptophan/kynurenine metabolism in the early stages of alcohol abstinence in chronic alcohol-dependent patients.

Effect of hypocaloric nutrition on inflammation markers and insulin sensitivity

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In some studies, duration longer than 4 weeks, it was shown that hypocaloric diet has a positive impact on inflammation markers and insulin sensitivity. The reduction of pro-inflammatory cytokines simultaneous with elevation of anti-inflammatory cytokines, leads to a reduction of chronic low-grade inflammation. Therefore the risk for developing diabetes type II, cardiovascular disease, cancer decreases and others. The aim of the study was to investigate if a short term (10 days) extreme hypocaloric diet already changes the inflammation markers adiponectin, leptin, IL-6, tumor necrosis factor-α (TNFα), high sensitive C-reactive protein (hsCRP) and the HOMA-IR. In this randomized controlled parallel group intervention study 38 participants (22 men/16 women) (BMI 28.95 ± 4.25 kg/m²; 52.81 ± 9.14 age) were assigned into two diet groups (Ø 590 kcal/day, Ø 940 kcal/day). The inflammation markers adiponectin, leptin, interleukin-6 (IL-6), TNFα, hsCRP and the homeostasis model assessment-insulin resistance (HOMA-IR) were tested at the beginning and the end of the study.

There was no significant reduction of IL-6, hsCRP, TNFα observed neither in the whole intervention group nor in the two diet groups. A significant reduction of leptin was observed in the whole intervention group and in the very low kcal diet group (whole group $p = 0.003$; very low kcal $p = 0.002$) but not in the low kcal diet group. The leptin/adiponectin ratio was significant lowered in the whole intervention group and in the very low kcal diet group (whole group $p = 0.028$, very low kcal $p = 0.009$) but not in the low kcal diet group. There was no significant change of the HOMA-IR in any of the study groups seen. In the whole intervention group and the low kcal group

the adiponectin level was significant lowered by the diet intervention (whole group p = 0.004; low kcal p = 0.003).

The body weight and the body fat was significant reduced in the whole study group (-2.77 ± 1.47 kg; resp. -1.95 ± 1.42%). After 10 days of caloric restriction there was also a significant reduction in body weight (very low kcal -2.75 ± 1.60, low kcal 2.81 ± 1.33 kg) and body fat (very low kcal -2.14 ± 1.49; low kcal -1.72 ± 1.34 %) in both intervention groups observed. Furthermore the BMI was significant reduced in the whole study group and in both diet groups (whole group -0.96 ± 0.45; very low kcal -0.98 ± 0.52; low kcal -0.92 ± 0.36 kg/m²). The muscle mass showed no significant difference in the whole study group (-0.18 ± -1.07 kg) and in both diet groups (very low kcal -0.33 ± 1.33; low kcal 0.8 ± 0.64 kg). After the diet intervention a positive correlation was seen between the body weight difference and the leptin difference in the whole study group (r = 0.375; p = 0.020) and the very low diet group (r = 0.592; p = 0.005). There was also a correlation between the leptin/adiponectin difference and the leptin difference observed in the whole study group (r = 0.920; p <0.001), the very low diet group (r = 0.907; p <0.001) and the low diet group (r = 0.933; p <0.001).

We conclude that a 10-day hypocaloric diet was sufficient enough to reduce significant the leptin and the leptin/adiponectin ratio. Nevertheless there was no significant positive change for IL-6, TNFα, hsCRP, HOMA-IR and adiponectin. To change the adipokines significant, the duration of the diet intervention has to be longer than 10 days. The leptin and the leptin/adiponectin ratio seem to be better markers for the change of the insulin sensitivity in the first days of caloric restriction, compared with the HOMA-IR.

Preoperative serum neopterin concentration and tryptophan degradation pattern in patients with late stage larynx carcinoma

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Head and neck squamous cell carcinomas (SCC) are highly immunogenic tumors. IL-12, interferon-gamma, IL-4, and IL-6 in laryngeal carcinomas have different patterns of gene expression, suggesting distinct pathways of Th1 and Th2 lymphocyte differentiation. Although surgery is a favorable prognostic factor for late stage laryngeal cancer, it does not impact on overall survival. In stage 3 and 4 larynx cancer, pathologic interactions between tumor and host immune cells might create an immunosuppressive environment that promotes tumor growth and protects the tumor from immune attack. Low tryptophan (Trp) levels or increased concentrations of its degradation products may be directly involved in diminished T-cell responsiveness. This phenomenon could be best explained by indoleamine 2,3-dioxygenase (IDO) activity which is estimated by serum kynurenine (Kyn)-Trp ratio. Aim of this study was to examine whether the late stage of laryngeal cancer enhances IDO activity and alters the increased frequency of serum neopterin concentration. Forty two male laryngeal SCC patients were included in this study. Thirty male cancer-free voluntary individuals with similar characteristics served as controls. The study was approved by local ethics committee. The patients were allocated into two groups; early stage (stage 1 and 2, n = 20) and late stage (stage 3 and 4, n = 22). Serum neopterin concentrations were determined by ELISA (BRAHMS, Berlin, Germany), while the serum Trp and Kyn were measured by high performance liquid chromatography. Serum IDO activity was estimated by Kyn to Trp ratio and subsequently the correlation between the serum neopterin and Kyn/Trp was examined. Although the neopterin levels of early stage cancer patients and healthy controls were similar (p >0.05), late stage group had significantly higher neopterin compared to both controls and early stage cancer individuals (both p <0.05). While the increased frequency of neopterin concentrations was 15 % in early cancer cases, at the late stage it was 59%. Preoperative Trp breakdown rate was significantly higher in late stage cancer patients. A highly significant correlation was estimated between the preoperative values of neopterin and Kyn/Trp in late stage cancer group (r = 0.555, p <0.01) and it was recognized as enhanced Trp breakdown rate which is most probably due to enhanced IDO activity. Postoperative evaluation of laryngeal cancer patients revealed that late stage group had higher serum neopterin compared to early stage patients, neither Trp nor Kyn levels showed a significant rise. Increased frequency of neopterin levels among the advanced cancer cases was an evidence of enhanced macrophage response to tumor antigen. Thus, increase in serum IDO activities of late stage laryngeal SCC patients may cause serious metabolic alterations which may facilitate the tumor progression irrespective of histo-pathological parameters.

Pathogen-derived volatile metabolites as breath markers of the microbial lung infections in the mechanically ventilated patients

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The routinely used microbiological diagnosis of ventilator associated pneumonia (VAP) is time consuming, requires invasive methods for collection of human specimens (bronchoscopy) and often might give the false negative results under certain circumstances (antibiotic treatment), proving the need for new diagnostic strategies in clinical settings, particularly in terms of accurate non-invasive detection of pneumonia outbreak. This may be achieved by monitoring the pathogen-derived volatile metabolites in the alveolar air of the patients. The development of such a breath test requires the identification of bacteria-derived biomarkers, hence the pneumonia causing microorganisms *S. aureus, P. aeruginosa, S. pneumonia, H. influenzae* and *C. albicans,* were investigated for the release or consumption of volatile organic compounds (VOCs) *in vitro* at densities found in the lungs of VAP patients.

A high number of diverse VOCs were released by all investigated species, even as early as 1.5 to 3 hours after inoculation, and their

concentration profiles ranging from ppt_v to ppm_v level were well correlated to the proliferation rate of the cultured microorganisms. Clear differences in bacteria-specific profiles of VOC production were found, particularly with respect to aldehydes which were only taken up by *P. aeruginosa* but released by *S. aureus* [1] and to hydrocarbons where entirely different VOCs of this group were released by *S. pneumonia* and *H. influenzae* [2]. On the other hand, noticeable similarities in VOCs metabolism were found for *S. aureus* and fungi *C. albicans* [3] being in accordance with a fact that these two species often coexist in infected lungs of VAP patients.

Subsequently, the longitudinal clinical study with mechanically ventilated individuals was performed to clarify whether *in vitro* results relate to the *in vivo* situation. The pneumonia was assessed on the basis of clinical (e.g. onset of fever, CRP level), microbiological (analysis of isolates) and imaging (CT or X-Ray) evidences. The exhaled air from the alveolar zone was collected directly from the respiratory circuit via endotracheal tube. The findings of breath analysis were referred to the clinical examination of a patient's health status, in particular to the level of C-reactive protein (CRP) considered to be a supporting (although unspecific) indicator of an overall infection/inflammation.

Our results demonstrate that the methodological platform developed (comprising breath samples collection, adsorptive preconcentration and GC-MS analysis) [4] proved to be suitable for application at bed-site in intensive care unit (ICU). The clinical study confirmed the presence of multiple pathogen-derived metabolites also in the alveolar air of VAP patients. In addition, the conformity of the concentration profiles of selected breath-VOCs with the course of pneumonia, as documented by CRP level, was observed for the donors examined at frequent time-points. Some of these VOCs were confirmed to be independent on smoking or inspired air pollutants [5], being potentially interesting as breath markers of pneumonia (e.g. 4-heptanone released by *C. albicans in vitro*, increased during the pneumonia and not related to smoking).

Therefore, we believe that the approach pursued here will enable non-invasive diagnosis of microbial lung infection in mechanically ventilated patients in the future.

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Immune response influences metabolism of aromatic amino acids

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Mood changes, depression and fatigue frequently develop in patients suffering from chronic inflammatory disorders such as infections, autoimmune diseases or cancer. The pathogenesis of such symptoms is still unclear. However, in the past years cytokine-induced alterations of biochemical pathways related to neurotransmitter biosynthesis came in the focus of intense research. The pro-inflammatory cytokine interferon-γ activates tryptophan-degrading enzyme indoleamine 2,3-dioxygenase (IDO) as an important antimicrobial effector pathway. This antiproliferative and pro-apoptotic leads to the suppression of immune responses as one side-effect, and due to its influence on the breakdown of tryptophan by IDO, toxic products accumulate and also the biosynthesis of neurotransmitter serotonin (5-hydroxytryptanmin, 5HT) is impaired [1]. The relevance of this observation for the neuropsychiatric status of patients has been already documented in inflammatory disorders or during cytokine treatment [2,3] and could also be induced in animal model systems [4]. In parallel to IDO, IFN-γ also stimulates enzyme GTP-cyclohydrolase I. In turn the production of neopterin in human monocytes/macrophages and of 5,6,7,8-tetrahydrobiopterin (BH_4) in other cells and cells of other species is markedly upregulated. BH_4 is cofactor for several aromatic amino acid monooxygenases that are involved in the biosynthesis of the neurotransmitters serotonin and the catecholamines dopamine, epinephrine (adrenaline) and norepinephrine (noradrenaline). This interaction represents another way how immunoregulatory cytokines may affect neurotransmitter biosynthesis, e.g., an impaired hydroxylation of phenylalanine as is indicated by and increased phenylalanine to tyrosine ratio (Phe/Tyr) and thus diminished activity of enzyme phenylalanine 4-hydroxylase has been observed in clinical conditions that are characterized by chronic immune activation and inflammation [5]. Future studies should be able to make use of Kyn/Trp and Phe/Tyr measurements to characterize patients more precisely regarding their potential treatment response towards selective monoamine reuptake inhibitors.

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Influence of nitrite content on HPLC measurements of tryptophan and kynurenine concentrations in human and murine serum

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The enzyme indoleamine 2,3 –dioxygenase (IDO) initiates tryptophan catabolism along the kynurenine pathway and is strongly associated with the activation of the immune system. The kynurenine to tryptophan ratio (Kyn/Trp) was found to provide a reliable estimate for the breakdown activity [1]. Thus, the monitoring of tryptophan breakdown can be done in a convenient way when the concentrations of the product kynurenine and its substrate tryptophan are measured. With this strategy a significant contribution of tryptophan breakdown in the development of neuropsychiatric

symptoms in patients with inflammatory disorders could be demonstrated. Moreover, accelerated tryptophan breakdown was found to predict outcome in patients with virus infections, with cardiovascular disease or with various types of cancer [2]. However, there is a relevant limitation when the measurement of the kynurenine and tryptophan levels is conducted in non-human or non-primate, e.g., murine serum specimens. This is due to differences of the nitric oxide ($NO^{\cdot}$) metabolism between human and other species, because in presence of high concentrations of nitrous acid or nitrites in an acidic milieu, diazotization of primary amides does occur [3]. Unfortunately, most HPLC methods for the analysis of tryptophan and kynurenine include precipitation of serum or plasma protein as a pre-analytical step. The deproteinization is done usually by the addition of trichloroacetic acid (TCA) or perchloric acid [4]. By the mixing of such acids with nitrite, nitrous acid is formed which can lead to the formation of N-nitroso-compounds finally leading to diazo-derivatives which may compose rapidly, only the aromatic forms of these products are more stable. Also amino-compound kynurenine undergoes such diazotization when specimens contain sodium nitrite and precipitation of proteins is performed by addition of, e.g., 2 µmol/L TCA. As a consequence, kynurenine is partly destroyed and the measured concentrations decline because the diazotizated product of kynurenine cannot be detected by HPLC measurement. In human specimens this effect is mostly negligible because nitrite concentrations in the serum or plasma are usually low, around 1-2 µmol/L. However, in animal model studies, e.g., in mice or rats, especially during inflammatory conditions nitrite concentrations can reach levels >20 µmol/L. Then, significant reduction of kynurenine concentrations becomes visible when serum protein is precipitated with strong inorganic acids. High nitrite levels in serum, plasma, cerebrospinal fluid or cell culture supernatants usually derive from high output of $NO^{\cdot}$ by cytokine inducible $NO^{\cdot}$ synthase (iNOS), the biological relevance of the enzyme in human monocyte-derived cells suffers from cofactor deficiency when induction of GTP-cyclohydrolase leads to the formation of neopterin at the expense of 5,6,7,8-tetrahydrobiopterin. Average nitrite levels in murine samples were found to be threefold higher as compared to human samples. Additionally we examined the effect of different concentrations of $NaNO_2$ added to human serum on the measured kynurenine content: low 2 µmol/L concentration $NaNO_2$ had only a minor effect on tryptophan and kynurenine levels but in the presence of 20 µmol/L $NaNO_2$ the kynurenine concentration measured in samples decreased significantly. In addition to the problem with the diazotization of kynurenine in the presence of $NO^{\cdot}$, it was also observed that expression and function of IDO is inhibited by $NO^{\cdot}$ [5]. Higher baseline $NO^{\cdot}$ production and thus IDO inhibition may explain why baseline tryptophan concentrations in murine/rat sera/plasma are considerably higher than in humans whereas kynurenine levels are lower. Thus, baseline tryptophan breakdown seems quite low in murine specimens. This is reflected also in culture media used for murine/rat cell lines (DMEM containing around 80 – 100 µmol/L tryptophan) as compared with human cell (RPMI containing around 30 – 35 µmol/L tryptophan).

In conclusion, because of the considerably higher nitrite levels in murine and rat serum/plasma samples, the diazotization of kynurenine and the potentially inhibition of IDO seems problematic and therefore the kynurenine assay with preanalytical TCA precipitation of proteins should be avoided. Rather alternative methodologies for deproteinization like the addition of acetonitrile or methanol or molecular sieves should be employed.

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IFN-γ ELIspot test *vs.* neopterin ELISA for diagnosis of *M. tuberculosis* infection

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For decades tuberculin Skin Test was a corner stone for the diagnosis of *Mycobacteria tuberculosis* infection. In recent years new assays with higher sensitive and specificity were introduced into clinical practice which indicate a present immunological response to *M. tuberculosis* based on the release of interferon-γ (IFN-γ) by isolated T cells from patients exposed to M. tuberculosis antigens in vitro [1,2]. These tests are thus termed IFN-γ release assays (IGRA or ELISPOT-Assay). A corner-stone in the immune response against M. tuberculosis is the increase formation of the TH-1 cytokine IFN-γ which subsequent activtation of anti-mycobacterial down-stream effects. Accordingly, pulmonary tuberculosis is associated with increased production of IFN-γ and the pteridine neopterin concentrations which is produced by macrophages upon IFN-γ stimulation [3]. In parallel, IFN-γ induced the formation of the enzyme indoleamine 2,3-dioxygenase (IDO) which leads to degradation of tryptophan to kynurenine and a number of subsequent metabolites [4]. Both, neopterin formation and IDO activity can be determined in peripheral blood mononuclear cells by an ELISPOST based assay. Within the assay, stimulated T-cells release IFN-γ which activates macrophages for neopterin production and tryptophan breakdown and neopterin formation is measured by ELISA (BRAHMS; Hennigsdorf, Germany) and Kyn/Trp as an index for the activity of IDO is monitored by HPLC. In this study, we compared the utility of the measuring neopterin and Kyn/Trp concentrations in supernatants with the detection of IFN-γ producing T-cells within the commercial ELISPOT protocol. Five sets of specimens (group #1: n = 24, group #2: n = 23, group #3: n = 21, group #4: n = 29, group #5: n = 43; total n = 139) were investigated. They underwent the regular ELISPOT pre-analytic and analytical test procedure: 100 µl of PMBC from blood of patients were incubated with *M. tuberculosis* antigens (ESAT-6 and CFP-10) overnight for routine testing, and IFN-γ formation by T- cells was measured semi-quantitatively after 24 hours with a ELISPOT plate reader. Supernatants of these analyses were used for neopterin and IDO determination. The *in vitro* responses of cells to the mycobacterial antigens were classified as negative, borderline, positive or non-specific. Of the 139 specimens, 98 (71%) were negative, 25 (18%) gave a positive result, 7 read-outs were borderline and 4 non-specific, another 5 specimens could not be interpreted. Neopterin concentrations ranged between 2 nmol/L (= lower limit of detection) and 9.2 nmol/L. Neopterin levels were insignificantly higher (mean 4.4. nmol/L) in ELISPOT positive specimens as compared to negative (mean 3.6 nmol/L) or borderline samples ones (3.5 nmol/L). Tryptophan concentrations were around 40 µmol/L in all 4 groups of individuals, kynurenine levels reached on average 0.53 µmol/L in the negative group but was even somewhat

lower in the positive group (0.46 µmol/L; not significant). Average Kyn/Trp was low in the positive group (8.5 µmol/mmol) and higher in the negative group (13.0 µmol/mmol; not significant). Comparing neopterin concentrations and tryptophan breakdown revealed a significant correlation in the whole group (kynurenine: rs = 0.214, p = 0.011; Kyn/Trp: rs = 0.207, p = 0.015) and in the largest subgroup #5 (kynurenine: rs = 0.437, p <0.01; Kyn/Trp: rs = 0.358, p <0.02).

In conclusion, an increase of neopterin production and Kyn/Trp was observed in some of the positive ELISPOT assay supernatants. There could be a potential for the diagnosis of tuberculosis by measuring neopterin and Kyn/Trp after the regular incubation within the ELISPOT test procedure. However, the test procedure needs to be optimized e.g., by the extension of the incubation time of PBMC. The increase of neopterin concentrations was found earlier to reach a plateau after 24-48 hours only [5]. However, this would increase the time required for completing the assay to 1 more day. Alternatively, the PCR detection of gene expression of IDO and/or GTP-cyclohydrolase I in the cellular compartment of the assay specimens could be feasible more rapidly.

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Preliminary results of serum neopterin levels and tryptophan degradation in food allergy

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Increase in neopterin concentrations and tryptophan degradation are common events following the activation of cellular immune system. In neurological, cardiovascular and autoimmune disorders, different types of malignancies and infections, high neopterin levels and tryptophan degradation have been reported to be well correlated with the severity of the diseases. Allergy is typically an immune-mediated disease with dysregulation of T-cell responses. Especially in children, there is limited number of studies investigating the role of tryptophan degradation and changes in neopterin levels in allergic disorders. In the present study, it was aimed to investigate the possible difference between patients with food allergy and healthy controls. For this purpose, neopterin was measured in 69 food allergy patients and 27 healthy controls without any allergic diseases were recruited into the study. Tryptophan and kynurenine levels were measured in 100 children with food allergy and 112 healthy controls. Serum neopterin levels were analyzed by ELISA (IBL, Germany) while serum tryptophan and kynurenine levels were detected by HPLC. Neopterin results were expressed as nmol/L and tryptophan degradation was presented as kynurenine to tryptophan ratio (µmol/mmol). Median (1st-3rd quartiles) levels of neopterin and Kyn/Trp were 5.99 (4.9-7.3) nmol/L and 47.7 (39.9-55.4) µmol/mmol in controls while 6.0 (4.9-7.9) nmol/L and 48.3 (36.8-55.6) µmol/mmol in children with food allergy. Kyn/Trp quotient that indicates the activity of IDO enzyme was lower in food allergic children [42.7 µmol/mmol (35.5-55.8)] than healthy controls [48.7 (40.6-59.9)] (p <0.01) while the other measured parameters did not show any statistical significance. The patients were further grouped according to their symptoms of asthma, allergic rhinitis, aeroallergen sensitivity, atopic dermatitis and perennial sensitivity. Due to the small number of patients, the results of subgroups are not presented in the abstract; they will be discussed with increased number patients. The preliminary data indicates that, in allergy group, increased tryptophan degradation is independent from neopterin concentrations and thus from Th-1 type immune response. Our ongoing studies will be more informative and precise about Trp degradation and Th-1 type response. Further detailed studies with increased number of subjects are needed to confirm our preliminary results and investigate the mechanisms in detail.

Neopterin, tryptophan breakdown and tyrosine metabolism during long-term post-withdrawal alcohol abstinence

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Previous studies have shown that tryptophan and tyrosine metabolism is disturbed during chronic alcohol consumption and acute alcohol withdrawal. We measured parameters of immune activation, tryptophan and tyrosine metabolism as well as alcoholism-related clinical parameters in post-withdrawal alcohol-dependent patients in the first 10-12 weeks of the abstinence. We present data of 54 patients (40 males, 14 females) who were admitted to 8-week post-withdrawal treatment. Clinical parameters and blood samples were collected at the first and eighth weeks of treatment. The patients abstained from alcohol on average 31 days prior to admission and reported 39 drinking days during the last 3 months. Depression and associated symptoms were screened by Beck Depression Inventory (BDI-2) and Montgomery-Asberg Depression Scale (MADRS). Craving-related symptoms were measured by Obsessive-Compulsive Drinking Scale (OCDS-d). The severity of alcohol dependence was assessed using Alcohol Use Disorder Identification Test (AUDIT). Detailed data upon alcohol consumption were collected by means of Timeline Follow Back scale (TLFB). The analysis of the data shows mildly elevated depression and craving scores at admission. Both depression and craving scores decreased significantly during the 8 weeks of post-withdrawal period as measured by BDI-2, MADRS and OCDS (p <0.001). There were significant increases of nitrite, kynurenine, kynurenine/tryptophan ratio and decreases of phenylalanine and tyrosine between the first and eighth weeks of treatment. A slight decrease of neopterin was not statistically significant. In the first week of treatment there was a significant correlation between neopterin and kynurenine to tryptophan ratio (Kyn/Trp) which disappeared in the eighth week. Selected BDI-2 items such as “irritability”, “disturbed sleep” and “fatigue” correlated significantly with the Kyn/Trp in the eighth week of treatment. Our data show that IDO-activation seems to play a role in tryptophan-kynurenine metabolism in the first weeks of alcohol abstinence in abstinent alcohol dependent patients. Additionally, there seems to be a relationship between

tryptophan-kynurenine metabolism and mild psychiatric symptoms during the first months of abstinence in alcohol dependent patients.

Lavender oil suppresses indoleamine 2,3 dioxygenase activity in human peripheral blood mononuclear cells

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Lavender essential oils are complex mixtures of mono- and sesquiterpenoid alcohols, esters oxides and ketones, with linalool, linalyl acetate, 1,8-cineole, β-ocimene, terpinen-4-ol and camphor as primary components. Lavenders and their essential oils have been used since centuries due to their antiseptic, antimicrobial and sedative effects. Lavender oil preparations have been suggested as gentle treatment options for several neuropsychiatric symptoms, due to their anxiolytic and mood alleviating effects. Today, the oil is often used for the treatment of conditions such as anxiety, restlessness, insomnia and depression [1]. Mood disorders are frequently associated with an increase of inflammation, and the metabolism of the essential amino acid tryptophan was found to provide a biochemical link between immunology and neuroendocrinology [2,3]. Tryptophan is the amino acid precursor of the neurotransmitter serotonin, and the tryptophan degradation product kynurenine can be metabolized to the neuroactive substances quinolinic and kynurenic acid. In this study, the interference of lavender oil with indoleamine 2,3 dioxygenase (IDO) mediated tryptophan catabolism was investigated in human peripheral blood mononuclear cells (PBMC). *In vitro* treatments of PBMC revealed a suppressive effect of lavender oil on mitogen induced tryptophan degradation and kynurenine formation at concentrations, were cell viability was only slightly affected. Additionally, the formation of the inflammation marker neopterin and of the cytokine and interferon-γ was partially inhibited. In unstimulated PBMC, tryptophan degradation was not influenced upon lavender oil treatment, while kynurenine formation and neopterin production were also decreased to a lower extent. Interferon-γ expression was not affected. Results indicate that lavender oil may contribute to the modulation of the serotonergic system by antagonizing cytokine-induced IDO activity.

Both tryptophan and kynurenine can cross the blood-brain barrier, thus peripheral levels of these can influence also brain tryptophan metabolism [4]. Lavender oil may slow-down tryptophan breakdown, which is often accelerated in patients suffering from inflammatory conditions and who also are at high risk to develop neuropsychiatric symptoms like anxiety and depression. Clearly further *in vivo* studies are warranted according to our data.

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Possible consequences of antioxidants in (alcoholic) beverages

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Excessive intake of alcohol is associated with an increased risk of a number of cancers: 3.6% of all cancer cases and 3.5% of cancer deaths worldwide can be related to increased consumption of alcohol. Alcohol has emerged as risk factor for a variety of cancer entities, such as breast cancer in women and cancers of the mouth, esophagus, pharynx and larynx, colon and rectum, liver, stomach and ovaries. For cancer of the pancreas, the relative risk of disease increases with augmented fat and alcohol intakes. However, it has been suggested that at least for pancreatic cancer, ethanol may be not directly involved in the etiology, but the effect could be due to the contents of some alcoholic beverages [1].

Alcoholic beverages like beer, wine and whiskey have been observed to suppress neopterin production and tryptophan breakdown in freshly isolated peripheral blood mononuclear cells (PBMC) in vitro [2-4]. Because also alcohol-free grape juice and beer were found to exert similar suppressive properties on PBMC, as do the stilbene resveratrol, the vitamins C and E, and other antioxidants [5], results point to a role of the antioxidant compounds in the beverages to be responsible for these effects. Data show a suppressive influence of the beverages on Th1-type immune response and production of cytokine interferon-γ (IFN-γ), a Th1-type cytokine with potent antitumoral and antimicrobial properties. This influence may relate to the benefit of some alcoholic beverages in moderate dosage to ameliorate inflammatory responses, which was observed in clinical conditions such as cardiovascular diseases. Moreover, inflammation associated breakdown of tryptophan, which is mediated by indoleamine 2,3-dioxygenase (IDO), was found to be accompanied by mood lowering [7]. Thus, the suppression of inflammation and IDO induction by alcoholic beverages could improve neuropsychiatric symptoms like depressive mood but also cognitive impairment and may in turn increase the risk of addiction. Alcohol itself is not active with this respect, but could improve the solubility and the gastrointestinal resorption of the antioxidant compounds. However, the suppressive influence of antioxidant compounds in beverages, natural products and preservatives like sulphites, could be of relevance due to the hampering of immunosurveillance and thereby increasing the risk for malignant disease and infections, and supporting tumor progression. Moreover, the selective inhibition of Th1-type immune response by antioxidant compounds may increase allergy risk and support weight gain [7].

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Antioxidant compounds suppress activity of diamine oxidase (DAO)

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The incidence of allergy and food intolerances has significantly increased during the past decades. About 1 % of the population presents with histamine intolerance or sensitivity, a suffering characterized by the appearance of various allergy-like symptoms [1]. Histamine is a biogenic amino acid that is contained in many foods, but can also be synthesized and released by a variety of cell types. In healthy people, histamine is degraded by the enzyme diamine oxidase (DAO) that is secreted into circulation upon stimulation. Inefficient degradation gives rise to high intestinal histamine, which is suggested to be responsible for the symptomatology, as histamine is a potent mediator of inflammation and allergic reactions [2]. Several studies already have discussed the involvement of exogenous antioxidants and "antioxidative stress" in the development of allergies [3,4]. To analyse the influence of selected compounds on DAO, DAO enzyme activity was examined by using radiolabelled putrescine dihydrochloride as substrate. Plasma samples from healthy human donors or DAO purified from porcine kidney were pre-incubated with curcumin, sodium benzoate or sodium sulphite. After liquid extraction, the reaction product Δ^1pyrroline has been measured. The exposure to the compounds resulted in a dose-dependent reduction of enzyme activity. If this *in vitro* effect of a suppression of DAO can be extrapolated to *in vivo*, it opens another possibility how overload with antioxidants may promote allergy development. This effect would be in addition to the suppression of Th1-type immune reactions and cytokines by antioxidant compounds which was demonstrated earlier for food preservatives, colorants, phytochemicals and drugs, thus promoting a shift of the Th1-Th2-type immune balance towards Th2-type immunity [5,6]. Although *in vitro* only, our results emphasize an additional effect to the suppression of Th1-type immune reactions and cytokines by antioxidant compounds, which was demonstrated earlier for food preservatives, colorants, phytochemicals and drugs [5,6], thus promoting a shift of the Th1-Th2-type immune balance towards Th2-type immunity.

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Antioxidant compounds suppress activity of diamine oxidase (DAO)

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The incidence of allergy and food intolerances has significantly increased during the past decades. About 1 % of the population presents with histamine intolerance or sensitivity, a suffering characterized by the appearance of various allergy-like symptoms [1]. Histamine is a biogenic amino acid that is contained in many foods and accumulates during prolonged storage. In healthy people, histamine is degraded by the enzyme diamine oxidase (DAO) that is secreted into circulation upon stimulation [1]. Inefficient degradation gives rise to great quantities of intestinal histamine that is suggested to be responsible for the symptomatology, as histamine is a potent mediator of inflammation and allergic reactions [2].

Several studies already have discussed the involvement of exogenous antioxidants in the development of allergies [3,4]. To analyse the influence of selected antioxidant compounds on DAO, DAO enzyme activity was examined by using radiolabelled putrescine dihydrochloride as substrate. After liquid extraction, the reaction product Δ1pyrroline has been measured in a betacounter (Sciotec, Tulln, AT). Plasma samples from healthy human donors or DAO purified from porcine kidney was pre-incubated for 30 min with curcumin, sodium benzoate or sodium sulphite. The exposure to the compounds resulted in a dose-dependent reduction of enzyme activity. If this *in vitro* effect of a suppression of DAO can be extrapolated to *in vivo*, it opens another possibility how overload with antioxidant compounds may promote allergy development. This effect would be in addition to the suppression of Th1-type immune reactions and cytokines by antioxidant compounds which was demonstrated earlier for food preservatives, colorants, phytochemicals and drugs, thus promoting a shift of the Th1-Th2-type immune balance towards Th2-type immunity [5,6].

Although *in vitro* only, our results further emphasize a strong interference of antioxidants with redox sensitive immunomodulatory enzymes and molecules. Whereas oxidative conditions support Th1 development, "antioxidative" stress is suggested to shift the balance towards allergic Th2-type responses.

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Tryptophan breakdown in patients with HCV infection relates to IL28B polymorphism

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Hepatitis C is a potentially fatal infectious disease, which affects millions of people worldwide. The standard treatment of chronic hepatitis C infection is a combination therapy with pegylated interferon-α (IFN-α) plus ribavirin. Recent studies have shown that single nucleotide polymorphisms (SNPs) adjacent to IL28B in the 19q13 region, in close proximity to three genes IL28A, IL28B, and IL29 encoding cytokines of the IFN-λ (i.e. type III IFN) family, predict spontaneous clearance of HCV infection as well as sustained viral response following IFN/ribavirin therapy among patients infected with HCV

genotype 1 [1]. Moreover, the Th1-type cytokine interferon-γ (IFN-γ) system seems to play a major role in determining outcome because the therapeutic outcome was associated with pretreatment plasma concentrations of the IFN-γ inducible protein (IP-10) [1,2]. Also elevated neopterin concentrations [2] and abnormal tryptophan [3] and phenylalanine metabolism [4] were described earlier in patients suffering from chronic HCV infection and such patients under IFN-α therapy. In this study, we investigated whether IL-28B SNP in 25 patients infected with HCV is related to the tryptophan breakdown rates. IL28B SNP distribution was as follows: 8 patients were homozygous C/C, 12 heterozygous C/T and 5 were homozygous T/T. Blood was drawn before patients received therapy with IFN-α, and in the sera tryptophan and kynurenine concentrations were measured by HPLC. As an estimate of the tryptophan breakdown rate, the kynurenine to tryptophan ratio (Kyn/Trp) was calculated. In addition, neopterin and nitrite concentrations were determined. There was a significant difference within the IL-28B polymorphism groups regarding the Kyn/Trp concentrations, patients with the C/C genotype were associated with the highest Kyn/Trp, patients with the T/T with the lowest Kyn/Trp ($p < 0.05$). And the differences were mainly due to alterations of kynurenine but not tryptophan concentrations. There was a also no difference regarding HCV plasma load and neopterin or nitrite concentrations (all comparisons did not reveal any statistical significance). The associations between neopterin and kynurenine and Kyn/Trp concentrations were only of borderline significance ($rs = 0.351$ and 0.352, both $p = 0.05$), so that the increase of Kyn/Trp cannot solely be ascribed to the activity of indoleamine 2,3-dioxygenase (IDO) in macrophages, IDO in hepatocytes and also tryptophan 2,3-dioxygenase (TDO) could play a major role in tryptophan breakdown during HCV infection. We conclude there exists a significant relationship between IL-28B polymorphism and tryptophan breakdown rates as expressed as Kyn/Trp. However, unlike IP10 concentrations which were usually lowest in patients with the C/C genotype, this genotype was associated with highest Kyn/Trp and T/T genotype was associated with lower Kyn/Trp. Further studies will be performed to find out whether these differences in tryptophan metabolism could relate to HCV clearance and could influence efficacy of and side effects of IFN-α therapy.

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Histamine intolerance - does it exist? A clinician's view

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The biogenic amine histamine [2-(4-imidazolyl)-ethylamine] is an important inflammation mediator in allergic diseases and asthma. During an allergic (= Th2-type immune) response histamine is released by mast cells and by basophil granulocytes. Histamine is vasoactive thereby decreasing blood pressure and increasing the permeability of the capillaries to white blood cells and some proteins. Histamine release occurs when allergens bind to mast-cell-bound IgE antibodies. Thus, the reduction of IgE overproduction may lower the likelihood of allergens finding sufficient free IgE to trigger a mast-cell-release of histamine.

Biosynthetically histamine derives from the decarboxylation of the amino acid histidine by the enzyme L-histidine decarboxylase (E.C. 4.1.1.22) and the degradation of histamine is achieved by N-metyltransferase (HNMT, E.C. 2.1.1.8) inactivating it in the brain and by diamine oxidase (DAO; E.C. 1.4.3.6) which is responsible for scavenging extracellular histamine [1].

Histamine exerts its effects by interaction with four specific histamine receptors. H(1)-antihistamines are now in clinical use as so-called inverse agonists for more than 70 years [2]. Second generation H(1)-antihistamines are relatively free from sedative and anticholinergic adverse effects and provide rapid onset of action for allergic rhinitis, conjunctivitis and urticaria treatment. Histamine also is able to modulate T cells by down-regulating Th1-type cytokines like interleukin 2 and interferon-γ (IFN-γ). On the same route, histamine suppresses neopterin production in the human myelomonocytoma cell line THP-1 [3].

Histamine occurs naturally in certain foods like fish, cheese, sea food, beverages like champagne, wine, beer, cider and other fermented drinks and spirits but also in sauerkraut and dried fruit. Other foods like bananas and tomatoes can stimulate the release of histamine from mast cells in the body. Normally DAO breaks down any histamine from a histamine-containing food, but in case of a low mucosal DAO activity in the jejunum, after intake of histamine-rich foods individuals may suffer from histamine intolerance with 'allergy-like' symptoms such as headache, rash, abdominal pain, diarrhoea, and vomiting. Unfortunately for the diagnosis of histamine intolerance serum activity of DAO turned out to be not useful marker [4]. Also molecular biological methods based on single nucleotide polymorphisms (SNPs) are not yet readily available. As a consequence, the pathophysiologic relevance of the histamine intolerance syndrome remains a little questionable. In the absence of an unequivocal test system a validated questionnaire and a validated skin prick test with histamine, measuring the delayed metabolism in skin is available for the diagnosis of histamine intolerance [4].

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Does stress in healthy individuals influence the phenylalanine-tyrosine pathway?

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Considering the current literature on distress in connection with depression a more and more established association becomes visible. Stress cannot just increase the impact of somatic diseases [1-3] it also can lead to changed immune competence which might

enhance depressive symptoms [4]. In patients with major depressive disorder (MDD) elevated levels of pro-inflammatory cytokines and other inflammation-related proteins have been found. Further data show that pro-inflammatory cytokines can have an influence on metabolic pathways which are known to be involved in the development of depression. Two pathways of current interest in stress- and depression-related research are the tryptophan and phenylalanine metabolisms.

Neopterin, Tryptophan and phenylalanine metabolites have been analyzed in 26 physically and mentally healthy students with a mean age of 23.9±1.7 years. A negative history of any psychiatric disorder, any recent infection or inflammatory disease, or any somatic condition affecting mood, the immune system, or the hematological system was required. The students were assessed twice, once without and once with chronic mental stress. The chronic mental stress condition was a major exam with an average preparation time of three months. The study protocol was performed at least 14 days in advance to the exam. At each time-point a standardized ergometer examination according to the Bruce protocol was used as an acute physical stress task. Blood was drawn four times, at rest and immediately after the acute physical stress condition. To assess past and present stressful life events, as well as emotional strain, the "List of Threatening Experiences Questionnaire" LTQ, the "Perceived Stress Scale 14" PSS-14 were used. Possible childhood adversities were assessed with the German version of the "Childhood Trauma Questionnaire" CTQ. In addition, the students had to rate their subjective amount of mental stress on a 10-point scale.

The values of the LTQ, PSS-14, and CTQ did not show any values above the cut-off at both evaluations. The students showed a mean subjective stress level above 7 on a 10-point range prior to the examination. For testing differences in the chosen parameters across distress conditions mixed linear model analyses were performed, including mental distress and physical distress and their interaction as fixed effects and subjects as random effects. Neopterin concentration was significantly increased due to physical stress. However, no significant effect was found on neopterin concentration due to mental distress. For the tryptophan/kynurenine pathway a significant interaction between mental and physical distress was found in tryptophan. Tryptophan levels were decreased due to physical distress both in the condition with and without mental distress. Likewise the kynurenine/tryptophan ratio was higher due to physical distress conditions. No significant main effect for mental distress was found for the ratio.

Main effects were found for mental and physical distress, both conditions being associated with lower tyrosine concentrations. No effects for mental or physical distress were found for phenylalanine concentrations. However, there was a significant main effect on the phenylalanine/tyrosine ratio due to mental distress condition.

These preliminary data indicate that acute physical and chronic mental distress has some impact on the metabolic pathways of tryptophan/kynurenine and phenylalanine /tyrosine. Further studies are warranted to elucidate these changes in greater detail and to gather more understanding for the interactions between stress conditions, immune activation and neurotransmitter pathways.

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Neopterin in normal CSF is brain-derived and not associated with blood CSF barrier dysfunction in non-inflammatory psychiatric diseases

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Analysis of cerebrospinal fluid (CSF) neopterin represents a relevant biomarker in a large variety of inflammatory and non-inflammatory neurological diseases. The source of neopterin in normal CSF has never been shown explicitly. In spite of its small size (molecular mass 253 Da) neopterin is exceptionally suitable for application of the molecular diffusion/CSF flow model to characterize its derivation. Only the investigation of normal controls and patients without an acute inflammation can provide unbiased data about increased CSF neopterin and its association with the blood CSF barrier function. Neopterin concentrations (ELISA, BRAHMS, Hennigsdorf, Germany) in CSF and serum are analysed for evaluation of variation propagation in controls (n = 26) and by reference to the albumin CSF/serum quotient (QAlb) in patients with psychiatric diseases (n = 44). CSF neopterin concentrations are predominantly brain-derived as inter-individual variation of CSF neopterin does not depend on serum neopterin concentration (coefficient of variation, CV-CSF = 9.7% < CV-serum = 24.5%) and as CSF neopterin concentrations in normal controls did not correlate with the albumin quotient, QAlb. In 31% of patients with schizophrenic and affective spectrum disorder there was a twofold increase of CSF neopterin concentrations, but there were no major differences between patients with normal QAlb or blood-CSF barrier dysfunctions. CSF neopterin is evaluated by its absolute concentration in CSF (cut off = 5.5 nmol/L). An increased CSF neopterin concentration has no common pathophysiology with the blood CSF barrier dysfunction.

Anaemia and immune activation in patients with angiographic coronary artery disease

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Iron metabolism is disturbed in patients with chronic diseases, anaemia is a frequent complication. In this study the relationship between coronary artery disease (CAD), iron metabolism and markers of immune activation and inflammation was investigated in

2040 individuals who underwent coronary angiography within the Ludwigshafen Risk and Cardiovascular Health study. 1571 patients with a stenosis of ≥20 % in at least one of 15 coronary segments were defined to have CAD and were compared with 469 controls. Patients suffering from CAD were older than controls ($p < 0.001$), and presented with higher inflammation and immune activation markers (leukocyte counts, neopterin and C-reactive protein (CRP) concentrations, all $p < 0.001$). Concentrations of iron ($p < 0.001$), tryptophan ($p < 0.001$) and folic acid ($p < 0.05$) were lower in both, male and female CAD patients compared to controls. Patients with more progressed CAD had lower haemoglobin concentrations (men: $rs = 0.106$, women: $rs = 0.201$; both $p < 0.001$). 346 individuals (221 men, 125 women, i.e. 17 % of the whole population) were anaemic, most of them suffered from anaemia of chronic disease (74.9 %). Patients with immune activation (i.e., cancer, auto-immune disease, infections or recent surgery, antibiotic treatment) suffered from anaemia more frequently (22.8 %). Haemoglobin concentrations were associated with parameters of iron metabolism, as well as with CRP, neopterin and tryptophan concentrations. Anaemia predicted a worse outcome of patients. Furthermore, also other parameters of iron metabolism (iron, transferrin, soluble transferrin receptor) and immune activation (CRP, neopterin) were predictive for the outcome of patients.

Serum tryptophan and phenylalanine metabolism in patients with Alzheimer's disease

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Increased serum neopterin concentrations and accelerated tryptophan breakdown, as is indicated by enhanced kynurenine to tryptophan ratio (Kyn/Trp), are well documented in a significant subgroup of patients with Alzheimer's disease (AD). Results showed that also peripheral immune activation may play a role in the pathogenesis of the disease [1,2]. More recently, elevated serum phenylalanine concentrations have been described in AD patients [3] which correspond well with observations made in patients with various inflammatory conditions such as infections, malignancy and under cytokine therapy, and thereby the phenylalanine to tyrosine ratio (Phe/Tyr) correlates with concentrations of immune activation markers like neopterin [4]. Increased Phe/Tyr indicates impaired activity of phenylalanine hydroxylase (PAH) which in turn is associated with disturbed biochemistry of dopamine, noradrenaline and adrenaline [4,5]. A relationship between Phe/Tyr and neopterin concentrations was also documented in AD patients [3] but there was no correlation of phenylalanine, tyrosine or Phe/Tyr levels and cognitive test performance of patients like mini-mental-test (MMSE) or clock drawing test (CDT) [3]. In this study, in the same 43 patients we additionally determined a neurobehavioral score (range 0-3). A mean value of 1.3 neurobehavioral abnormalities was observed in the patients, and the scores correlated with MMSE ($rs = -0.374$, $p < 0.01$), CDT ($rs = -0.372$, $p < 0.01$) and tryptophan concentrations ($rs = -0.274$, $p < 0.05$). However, neither phenylalanine nor Phe/Tyr concentrations correlated with neurobehavioral score. When comparing medications with the concentrations of various laboratory markers, Phe/Tyr concentrations were higher in those who were treated with acetylcholinesterase inhibitors ($U = 2.100$, $p < 0.05$), and those who received selective serotonin reuptake inhibitors had lower neopterin concentrations. We conclude that neither serum phenylalanine nor Phe/Tyr concentrations in AD patients were related to the neurobehavioral score. However, the score used provides only a rough estimate of the neuropsychological situation of the patients and only more sophisticated questionnaires may be able to allow more solid conclusions. Thus, still more extended studies with additional parameters are needed to elucidate a potential link between the disturbed phenylalanine metabolism and neurobehavioral symptoms including delirium in AD patients as such an association was reported for low tryptophan (6).

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Risk profiles in obese juveniles versus adults - the STYJOBS/EDECTA cohort

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Obesity is dramatically increasing. Atherosclerosis, a major consequence of obesity, starts early in life and often results in cardiovascular disease. The STYrian Juvenile OBesity Study (STYJOBS) is a prospective study to improve the understanding of AS in obesity by investigation of the "non-biased" early phase. EDECTA (Early DeteCTion of Atherosclerosis) extends STYJOBS up to the age of 65 years. The aims of this project are (i) Identification of "individual high risk patterns" for cardiovascular disease in young, and middle aged obese people by linking biomarkers, adipose tissue topography, early vascular changes, and clinical data and (ii) to establish a serum/plasma/DNA/RNA resource of obese, and normal weight young and middle-aged people for advanced research of atherosclerosis and metabolic risk profiles in obesity. Applicated methods comprise the analysis of routine lab, biomarkers, oxidative/nitrosative stress markers, carotis sonography [intima-media thickness (IMT)], and adipose tissue topography. So far we investigated 600 obese (age range 5-55 years) and 350 normal weight, age matched healthy controls. Intended, n=1500. Obese patients exhibit an increased IMT accompanied by a low grade inflammation as early as in the beginning of the 2nd life decade [1,5,7]. The ratio between HMW and total adiponectin is significantly decreased in obese patients whereas the LMW / total adiponectin ratio is increased [1,5,6]. The HMW / total adiponectin ratio correlated significantly negatively, and the LMW / total adiponectin ratio significantly positively with the IMT [5,6]. Multiple regression analysis of body measures and all other lab parameters showed the strongest correlation between

HMW adiponectin and carotid IMT [1,5,6]. Truncal obesity was negatively associated with HMW adiponectin in juveniles and adults [5]. Further, while obese juveniles showed a decreased kynurenine to tryptophan ratio (KYN/TRP; an indirect sign for Th2-type cell activation), adult obese participants had an increased KYN/TRP indicating upcoming Th1-type immune activation in advanced phases of obesity [Obesity submitted]. Our data underline the close relationship between obesity, inflammation, and pre-atherosclerosis [1-7]. This pathology, and the dysregulation of adipokines is closely linked to the SAT-tissue topography [4-6]. We provide evidence that preatherosclerosis in early phases of obesity is yet associated with altered oligomerisation of adiponectin subfractions [4-6]. Further, the early and advanced phases of obesity differ markedly from each other [1]. Thus, factors that influence a switch of „immunologic quality of inflammation" towards sustained vascular burden should be investigated more in depth in future studies.

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Urinary neopterin during neoadjuvant chemotherapy in patients with breast cancer

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Neoadjuvant chemotherapy is the treatment of choice in patients with locally advanced breast carcinoma. In addition to increasing the disease control rate, the neoadjuvant chemotherapy allows for prognostic stratification. The activation of the immune system may play an important role in the mechanism of action of systemic chemotherapy. In earlier studies, it was demonstrated that the infiltration of the tumor by lymphocytes predicts the response to neoadjuvant treatment, and that administration of neoadjuvant chemotherapy activates the immune response. In the present study, urinary neopterin was examined in 31 patients treated with neoadjuvant chemotherapy. Baseline neopterin concentrations were higher in patients with triple negative breast cancer ($p=0.06$). Fluctuations of urinary neopterin levels were observed during systemic treatment, including the treatment with trastuzumab in combination with chemotherapy. In conclusion, fluctuating urinary neopterin concentrations in patients with breast cancer during the course of neoadjuvant treatment indicate the presence of immune system activation.

The role of heme oxygenase 1 in regulating iron homeostasis and innate immune response to Salmonella Infection

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Macrophages play an essential role in the containment and elimination of microbes. Heme oxygenase-1 (HO-1, *hmox1*) the enzyme cleaving heme to ferric iron, billiverdin and carbon monoxide, is involved in the regulation of stress response, iron homeostasis and host pathogen interactions. Thus, we studied herein the regulatory effects of this gene in the course of Salmonella infection in macrophages and associated changes of iron homeostasis and innate immune function.

We used the murine macrophage cell line RAW264.7 and constructed lenti-virus based tetracyclin inducible shRNA knock downs of the *hmox* gene. For infection experiments we used the intracellular bacterium *Salmonella enterica* serovar Thyphimurium. Upon knock down of *hmox* in RAW264.7 cells we observed alterations of iron gene expressions, most strikingly with an up-regulation of the iron exporter ferroportin-1 (FPN1) mRNA and protein expression. To study the relevance of these observations for host response to infection we infected macrophages with *Salmonella*. Interestingly, the knock down of HO-1 reduced the survival of Salmonella in macrophages whereas it had no effect on pathogen uptake. The improved pathogen control could be traced back to reduced iron availability for intramacrophage bacteria and partly improved innate immune function as a consequence of increased ferroportin expression and intracellular iron restriction. Our data suggest that HO-1 plays a central role in host responses towards intracellular pathogens by modulating cellular iron homeostasis in macrophages and anti-bacterial immune effector pathways.

Influence of sodium sulfite on IDO mediated tryptophan catabolism and chronic allograft vasculopathy

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The food preservative sodium sulfite (E221) has recently been shown to abrogate Th-1-type induced indoleamine 2,3-dioxygenase (IDO) activity *in vitro*. However, its influence on IDO-mediated allograft survival *in vivo* remains unclear. Here we studied the effects of E221 on IDO mediated tryptophan degradation and chronic allograft vasculopathy in a murine aortic transplant model. C57BL/6 mice received BALB/c vascular allografts (no treatment, group 1). Recipients were

either treated with sodium sulfite (0.1 mol i.p. daily; group 2) or sodium sulfate (0.1 M i.p. daily; group 3). Untreated (group 1) and syngeneic (group 4) transplants served as controls. Serum enzyme activity of IDO was analyzed by HPLC. Histopathology (H&E, Elastica van Gieson) and immunohistochemistry (for smA, CD4, CD8, Foxp3, IDO) of allografts was performed at pod 50 and 100. C57BL/6 mice receiving BALB/c vascular allografts (group 1) displayed increased IDO activity over the entire observation period (pod 5 - 100) and vessels showed distinct signs of chronic allograft vasculopahty (neointima proliferation, endothelial damage) when compared to syngeneic controls ($p < 0.05$). Sodium sulfite treatment (group 2) significantly suppressed IDO activity, tryptophan degradation and hence kynurenine formation ($p < 0.05$). Furthermore, sodium sulfite treated animals showed significantly higher rejection scores when compared to sodium sulfate (group 3) treated animals ($p < 0.05$). This study provides first *in vivo* evidence that sodium sulfite inhibits IDO-mediated tryptophan catabolism and thereby aggravates chronic allograft vasculopathy in a murine aortic transplant model.

Neopterin and cholesterol concentrations and blood pressure in repeated voluntary blood donors

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Neopterin is not only a marker of inflammation and immune system activation but also an active participant in cardiovascular disease [1,2]. Based on epidemiological data a serious number of blood donors are at risk for cardiac diseases. Elevated blood pressure as well as elevated cholesterol levels are major risk factors for developing cardiovascular disease. To investigate an assumed relationship between blood pressure, total cholesterol and neopterin levels we investigated the results 1805 blood donors, everyone having donated whole blood twice in 2012, i.e. 3810 investigated donations. The time between first and second donation was 6 month (±2 weeks).

Blood pressure was measured immediately before blood donation (DinamapTMPlus, Sanitas), serum neopterin levels were determined every donation, assay was performed and interpreted according to the manufacturers' instructions (BRAHMS Neopterin Screening EIA, Thermo Scientific, Hennigsdorf, Germany) Measurement range is from <2 nmol/L to >50 nmol/L. Total cholesterol was performed on Dimension and interpreted according to manufacturers' instructions (Siemens Healthcare Diagnostics Nework, Germany).

For statistical analysis by Fishers exact test blood donors were first grouped by age (group 1: 31-40a, group 2: 41-50 y, group 3: 51-60 y and group 4 older than 60 y). Age distribution of the 1805 donors was group 1: 391 (21.7%), group 2: 372 (20.6%), group 3: 708 (39.2%) and group 4: 334 (18.5%). Age distribution showed that more than half of the donors are older than 50 y, indicating one of the problems to come near in blood supply within the next 10 years. As expected and as described in the literature the mean values of all investigated parameters (neopterin, total cholesterol, blood pressure) increased with rising age group.

First in blood donor screening robustness of a test method is of great importance, hence we were highly interested in the correlation of neopterin level in the 1805 paired donations. Correlation of both neopterin levels was highly significant ($p < 0.001$) in every age group, with coefficient of correlation ranging from 0.689 to 0.720. This means an excellent correlation for a parameter being influenced by innumerable aspects, indicating that maybe even slight changes are essentially caused by the donor and not by the test method. There was no significant correlation between blood pressure and neopterin levels in any of the four different age groups, but there was a significant positive correlation between blood pressure and total cholesterol in group 1 and group 2 ($p < 0.001$ and $p = 0.001$ resp.) which disappeared in group 3 and group 4. The most interesting result was a significant negative correlation between total cholesterol and neopterin level in group 1 and group 2 ($p = 0.001$ and $p < 0.001$ resp.) which is not the case in group 3 and 4.

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PSA, neopterin and tryptophan breakdown in patients with cancer of the prostate

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Prostate cancer is the most frequent cancer disease in Austrian men. Forty-nine men with elevated (= above previously defined age-dependent cut-off values ranging between 1.25 and 3.75 µg/L) serum prostate-specific antigen (PSA) and tumor-free prostate biopsy (benign patients), 101 men with by the same cut-off values elevated serum PSA and tumor positive prostate biopsy (first-diagnosed cancer of the prostate) and 50 men with a recurrence of the tumor at a median time of 7.2 (range: 2.7 to 17.5) years after radical prostatectomy were investigated to evaluate the diagnostic and prognostic potential of the tumor marker PSA [1,2] and serum neopterin [3,4] and tryptophan breakdown [5], two markers of Th_1-type immune response. PSA concentrations were lowest in benign patients (median, range: 3.5, 1.0 to 17.9 µg/L), higher in first-diagnosed prostatic cancer (median, range: 5.0, 1.2-32.1 µg/L) and highest in those with a recurrent cancer (median, range: 36.9, 21.2-2926.0 µg/L). Thirty-nine percent of the men with benign conditions but 65% of those with first-diagnosed cancer had PSA concentrations above 4 µg/L which resulted in a diagnostic sensitivity of 65% and a specificity of 63%. Similar to PSA, serum neopterin and kynurenine per tryptophan ratios (Kyn/Trp) were lowest in benign patients (median, range: neopterin 4.9, 2.6-16.2 nmol/L; Kyn/Trp 53.5, 24.7-96.6 µmol/mmol), higher in first-diagnosed prostatic cancer (median, range: neopterin: 5.3, 3.6-38.8 nmol/L; Kyn/Trp 56.3, 20.3-99.8 µmol/mmol) and highest in those with a recurrent cancer (median, range: neopterin 9.9, 4.2-100.0 nmol/L; Kyn/Trp 67.1, 38.8-402 µmol/mmol). Sixteen percent of the benign prostate men, but 18% with first-diagnosed cancer showed neopterin concentrations above the reference range resulting in a diagnostic sensitivity of 18% and a specificity of 84%. There was a significant correlation between PSA concentrations and tumor size (Spearman's rank correlation: $r_s = 0.495$; $p < 0.0001$) and Gleason scores (Spearman's rank correlation: $r_s = 0.375$; $p = 0.0001$), whereas no significant association could be found between neopterin concentrations and Kyn/Trp and tumor size or Gleason scores (all $p > 0.05$). In the group with a recurrence of the prostate cancer 28 died from prostate cancer after

a median time of 1.0 (range: 0-12) years. Those with PSA, neopterin or Kyn/Trp above the third quartile point of the observed parameter distribution, showed a statistically significant shorter survival than those with values below it (Breslow-test: PSA: p = 0.033; neopterin: p <0.001; Kyn/Trp: p = 0.017). From the data obtained neither neopterin nor tryptophan breakdown proved as reliable for diagnosis of prostate cancer. The use of PSA for prostate cancer screening is still a point of discussion. In contrast to neopterin or Kyn/Trp, PSA was correlated with tumor size and tumor differentiation. The prognostic impact of PSA, neopterin or Kyn/Trp concerning survival expectations has to be further investigated.

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Linkage of iron homeostasis to immunity and infection

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The control over iron availability is of central importance in host-pathogen interaction because mammalian cells and microbes have an essential demand for the metal, which is required for many metabolic processes and for microbial pathogenicity [1]. In addition, cross-regulatory interactions between iron homeostasis and immune function are evident. Cytokines and the acute phase protein hepcidin affect iron homeostasis leading to the retention of the metal within macrophages. This is considered to results from a defense mechanism of the body to limit the availability of iron for extracellular pathogens while on the other hand the reduction of circulating iron results in the development of anemia of inflammation [2]. Opposite, iron as well as the anemia inducible hormone erythropoietin affect innate immune responses by influencing IFN-γ mediated (iron) or NF-κB inducible (erythropoietin) immune effector pathways in macrophages [1, 3]. Thus, macrophages loaded with iron lose their ability to kill intracellular pathogens via IFN-γ mediated effector pathways such as nitric oxide (NO) formation. Accordingly, macrophages invaded by the intracellular pathogen *Salmonella typhi murium* increase the expression of the iron export protein ferroportin thereby reducing the availability of iron for intramacrophage bacteria while on the other side strengthening anti-microbial macrophage effector pathways via increased formation of NO or TNF-α [5]. In addition, certain innate resistance genes such as natural resistance associated macrophage protein function (NRAMP-1) or lipocalin-2 exert part of their antimicrobial activity by controlling host and/or microbial iron homeostasis [6,7]. In a line of this, pharmacological modification of cellular iron trafficking e.g. by the calcium antagonist nifedipine [7] enhances host resistance to intracellular pathogens via limitation of iron availability [8]. Thus, the control over iron homeostasis is a central battlefield in host-pathogen interplay influencing the course of an infectious disease in favor of either the mammalian host or the pathogenic invader.

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Tetrahydrobiopterin prevents murine isograft from brain death exacerbated ischemia reperfusion injury.

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Brain death (BD) is associated with an immunological priming of grafts and is thought to exacerbate ischemia reperfusion injury (IRI). Recently we were able to demonstrate that the nitric oxide synthase co-factor tetrahydrobiopterin (BH4) abrogates IRI following murine pancreas transplantation. Herein we assessed the impact of BD on IRI and tested the therapeutic potential of BH4. Pancreas transplantation was performed between syngeneic C57BL/6 mice. Animals were followed for 3h after BD-induction. Experimental groups included: non-treated BD donors, pre-treatment of BD donors with 50mg/kg BH4, ventilated non-treated donors (sham group), non-brain death donors (living donors). Following 2h of reperfusion, graft-microcirculation (functional capillary density, FCD; capillary diameter, CD) as well as cell viability were assessed by intravital fluorescence microscopy. Parenchymal graft damage was histologically assessed, BH4 levels were determined by HPLC and mRNA expression of inflammatory markers was measured by Real Time qPCR. BD had dramatic impact on pancreatic microcirculation as highlighted by reduced FCD and CD values when compared to controls (p <0.05). Moreover BD induced intragraft mRNA expression levels of IL-1ß, TNFα, IL-6 and ICAM-1. In contrast BH4 treated grafts displayed significantly improved microcirculation as reflected by significantly higher FCD and CD values (p <0.001, respectively). BD impacted cell viability following reperfusion, whereas BH4 treated grafts displayed similar percentages of viable cells as non brain death controls (p <0.001). Parenchymal damage was significantly more pronounced in organs from BD donors when compared to controls (p <0.05). Pre-treatment with BH4 however significantly ameliorated parenchymal damage in organs from BD donors (p <0.05). In conclusion,

This study provides in vivo evidence that BD aggravates IRI after experimental pancreas transplantation. Donor pre-treatment with BH4 offers a novel therapeutic option in this setting.

Changes in neopterin levels and kynurenine pathway in bitumen workers

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Bitumen (asphalt), widely used in road paving, roofing and many other surface applications, consists of a complex organic mixture including polycyclic aromatic compounds (PACs) that result from the incomplete combustion of materials such as coal. Workers in these occupations have been shown to have exposure to PACs in asphalt vapors and fume via inhalation and/or dermal absorption. The International Agency for Research on Cancer (IARC) has classified extracts of steam-refined and air-refined bitumen in Group 2B as possible human carcinogens. Measurements of bitumen compounds are challenging due to the complex nature of its vapours and aerosols. The aim of this study was to investigate neopterin levels as a possible exposure biomarker and to evaluate tryptophan degradation in bitumen workers. Eighty-four bitumen workers who admitted to Ankara Occupational Diseases Hospital for routine check-up were included in this study while 61 healthy subjects recruited as a non-exposed group. Urinary neopterin levels of bitumen workers were 214 ± 106 µmol /mol creatinine (mean ± SD) while 136±38 µmol/mol creatinine for control group ($p = 0.001$). Kynurenine to tryptophan ratio of exposed and control groups were 47.11 ± 11.88 µmol/mmol and 31.56 ± 9.72 µmol/mmol, respectively. Kynurenine/tryptophan was statistically higher in bitumen workers ($p = 0.001$) while no difference was observed in tryptophan levels compared to controls. Even though the workers have no diagnosis related to bitumen exposure, these results may be concluded that neopterin has a potential to be used as an early exposure biomarker in bitumen exposure. Besides, induction of tryptophan degradation leads to increase in kynurenine metabolites. Over production of these metabolites may result in changes in cellular level via different biochemical pathways.

Neopterin: biomarker for chronic lymphocytic leukemia?

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Urine and serum neopterin concentrations are well known to be increased in patients with hematological neoplasias [1]. Moreover, in these patients neopterin concentrations are of significant predictive value [2,3]. Therefore, the monitoring of urinary neopterin concentrations was proposed as a useful marker for the assessment of patients with myeloid neoplasias [1]. In this study, we extended our investigation of 2011/12 [4] whether plasma neopterin concentrations are changed in patients with chronic lymphocytic leukemia (CLL) and whether the determination of neopterin concentrations may help as a guide for the physician in taking care of patients. From 265 patients (142 males, 123 females, median age 62 years, range: 23-88 years) 1146 plasma specimens were obtained at diagnosis and during follow-up. Neopterin concentrations were measured by ELISA (BRAHMS; Hennigsdorf, Germany). Compared to the reference range of healthy controls (mean ± SD: 5.3 ± 2.7 nmol/L) significantly higher neopterin levels were observed in CLL patients (31.0 + 35.6 nmol/L, median 18.3 nmol/L, range 3.0 – 250 nmol/L; $p < 0.001$), and there was no difference of neopterin concentrations between, males (29.9 ± 36.2 nmol/L) and females (32.3 ± 34.7 nmol/L; not significant). Data obtained during follow-up seem to indicate additional value of neopterin measurements in the judgment of patients and to indicate their immune status [5]. Further analyses of the data are directed to investigate a potential predictive value of neopterin concentrations in CLL patients.

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Immunosuppressive properties of bisphenol A

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Bisphenol A (BPA), a widely used plastic monomer and plasticizer, is in the focus of consumer protectors because of its genotoxic and carcinogenic potential. Moreover, BPA may also be involved in development of allergy and weight gain and thus in the obesity epidemic [1]. BPA is also a chemical with well-known antioxidant chemical property. Earlier an influence of several antioxidant compounds, plant extracts and medications to inhibit responsiveness of human peripheral blood mononuclear cells (PBMC) has been documented [2]. This study in the PBMC model system investigated the influence of BPA on mitogen-induced production of interferon-γ (IFN-γ), neopterin and tryptophan breakdown, which were used as sensitive readouts for the activation status of cells [2]. We observed significant and dose-dependent suppressive activity of BPA on T-cell responsiveness and the stimulation of cytokine cascades in vitro. IFN-γ production as well as neopterin production and tryptophan breakdown were diminished, all these immunobiochemical changes are indicative for Th1-type immune response.

Antioxidant compounds usually are looked upon very favourably as long as vitamins like C and E or other food components like polyphenols, carotenoids, or coenzyme Q10 are considered [5]. An anti-inflammatory property of such compounds seems evident at least from in vitro studies, and it is extrapolated from findings that they may be able to explain the cardioprotective and health- and life-prolonging effect of a diet rich in antioxidants. On the other hand, the list of dietary compounds with anti-inflammatory properties includes also chemicals and food supplements like preservatives and colorants [3,4]. Inhibition of Th1-type immunity by BPA could be relevant for an increased risk of allergy development when BPA suppresses Th1 type cytokine IFN-γ and thereby shifts immune cells away from a Th1-type towards a Th2-type cytokine pattern [3,4]. The inhibitory effect of BPA on T-cell activation and the formation

of IFN-γ is also critical for immunosurveillance and may relate to an increased risk of tumor cell proliferation upon exposure to BPA. Recently also an association was observed between urinary BPA concentrations and obesity and one cannot rule out the possibility that BPA is involved in weight gain. BPA also could indirectly contribute to weight gain and obesity in exposed individuals when it interferes with leptin signalling as other antioxidant compounds were shown to do: recent in vitro studies using NIH 3T3 adipocytes demonstrated that antioxidant food preservatives sodium sulphite and benzoate as well as colorant curcumin significantly suppressed the production of leptin [6], a key element in the long-term regulation of food intake and body weight homeostasis. Thus, BPA like other antioxidant compounds might also contribute to insufficient leptin production and as a consequence down-regulation of food intake is delayed.

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A critical comparison of creatinine-based GFR-formulae with kinetically measured glomerular filtration rate

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Exact measurement of kidney function requires kinetic analysis of time-dependent blood concentrations of suitable marker substances. As these methods are time-consuming and laborious, for routine purposes simple formulae have been developed which – based on serum creatinine concentrations – allow rapid estimation of glomerular filtration rate (GFR) as a measure of renal function. We compare nine GFR formulae with kinetic clearance measurement using the inulin-like polyfructosan sinistrin. Creatinine and sinistrin serum concentrations were determined in 161 patients with quite diverse diseases. Sinistrin-based kinetic GFR measurement is performed using the standard two-compartment model of pharmacokinetics. Notably, this model is mathematically formulated by two coupled differential equations which describe the time-dependent concentrations of the marker sinistrin in a well perfused central body compartment and a less perfused peripheral compartment. The differential equations contain as parameters the kinetic constants describing the distribution of the marker between the two compartments, the elimination rate constant and the volume of the central compartment. The parameters are determined by fitting the model equations to the time-dependent marker concentrations in the blood of patients after having received a bolus injection of the marker substance. From the fitted parameters, many kinetic and functional properties of the kidneys can be obtained. Statistical comparison of the creatinine-based GFR estimations and the kinetic clearances are done by linear regression and the Bland-Altman method. The abilities of the formula-based GFR estimations to discriminate between patients with normal, mildly impaired and severely restricted renal function are judged by classical evaluation methods of clinical chemistry (diagnostic sensitivities and specificities) as well as by information theoretical analysis.

All formula-based GFR values are strongly correlated with each other, but their agreement with kinetic GFR measurements is only moderate and particularly poor in patients with normal and mildly disturbed renal function. The correlations are, however, better in the range of severely impaired kidney function. Similarly, GFR formulae are of limited value to discriminate between normal and mildly disturbed renal function; somewhat better results are obtained for the discrimination between severely reduced GFR and normal/mildly disturbed kidney function.

Formula-based GFR estimations yield sufficiently precise information only in case of severe reduction of renal function. The reason for the failure of these formulae to describe sufficiently exactly situations with better renal function is the hyperbolic (inverse) relationship between serum creatinine concentrations and GFR: in the range of creatinine levels below about 1.5 mg/dL, a broad spectrum of normal and mildly decreased GFR values can be found ("creatinine-blind range"). When precise discrimination between normal and mildly restricted renal function is required, kinetic measurement of GFR remains the method of choice. Such situations can arise, e.g., in the selection of live kidney graft donors as it is known that even a mild renal impairment in the donors increases the risk of subsequent graft loss considerably.

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Increased IDO activity and neopterin concentrations in euthymic overweight bipolar patients

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Bipolar disorder (BD) is a severe and chronic mental disease characterized by episodic, recurrent pathological disturbance in mood ranging from extreme elation to severe depression. The excess in overweight (up to 70%) and metabolic syndrome in patients with BD is amply documented [1] and associated with an excess mortality, increased frequency of episodes, shorter periods of euthymia and more suicide attempts [2-4]. This is the first preliminary report of our BIPFAT study exploring shared pathophysiologic pathways between overweight and BD. We investigated the dysregulation of tryptophan-kynurenine metabolism in euthymic BD patients compared with healthy controls. Specifically, we focussed on the

differences between overweight BD patients (n = 56) and overweight controls (n = 22), to further elucidate common pathways underlying the increased prevalence of overweight in BD and the worsening of prognosis in overweight BD patients.

There are two important findings in this study: (1) Blood kynurenine levels, as well as the kynurenine to tryptophan ratio (Kyn/Trp, an estimate of the activity of indoleamine 2,3-dioxygenase, IDO) are increased significantly in the euthymic BD patients in general and even more in the overweight BD patients in comparison to overweight controls. Interestingly, tryptophan levels were significantly decreased only in normal weight BD patients. We found no influence of the stage of illness or duration since the last affective episode on tryptophan, kynurenine and Kyn/Trp concentrations. (2) Neopterin increased in overweight BD patients in comparison to normal weight patients and demonstrates increased cellular (Th-1 type) immune activation which is further confirmed by our finding of the increased IDO activation. Moreover, we found increased neopterin concentration in advanced stages of BD. Similar reports were seen in patients with Alzheimer's dementia, as increased neopterin concentrations correlated directly with the cognitive decline [2]. The increased neopterin in BD may therewith account for neurodegenerative processes upon chronic inflammation.

We conclude that the increased IDO activity as well as increased neopterin levels in BD may be an indirect mediator of immune-mediated inflammation which has already been shown to be involved in the development of atherosclerotic vascular lesions in overweight healthy adults. In BD this may account for the high prevalence of medical comorbidities and increased mortality. Importantly, research in this area is needed to develop fundamental personalized strategies in prevention and treatment of BD.

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Detection of trimethylamine in haemodialysis patients

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Trimethylamine (TMA) is a volatile short chain N-containing compound with fishy odor which can derive from diet directly or as a metabolic product. It has been already recognized as potential marker for end-stage renal disease causing so called "uremic breath" in patients suffering in renal failure. Thus, detection of TMA from exhaled breath might be a promising approach in diagnosis or monitoring of renal disease stage. Chronic kidney disease (CKD) is often diagnosed late because its initial stage is usually asymptomatic. During the last decade the number of patients suffering from chronic kidney diseases (CKD) has increased, therefore an early diagnosis and appropriate treatment is necessary in order to prevent end-stage renal disease (ESRD). We measured TMA in expired air of renal diseases patients before and after haemodialysis using two techniques: gaschromatographic analysis coupled with mass spectrometer (GC-MS) and proton-transfer-reaction time of flight mass spectrometer (PTR-TOF-MS). For the GC-MS analysis thermal desorption was used in order to enrichment of samples. TMA concentration in exhaled air of dialyzed patients was higher before than after dialysis. In contrast to GC-MS, PTR-TOF-MS permits fast, on-line analysis of breath volatiles without any preconcentration steps. Using the great mass resolution of $\Delta m/m \sim 1/5000$, TMA (60.08 m/z) could be separated successfully from the ^{13}C-acetone isotopomer (60.05 m/z) with a mass difference of 0.03 m/z. Similarly like using GC-MS method we found higher TMA concentration values in breath samples before dialysis than after or during the treatment for every examined CKD patient. Our investigation will be developed towards the elaboration of reliable breath tests as new diagnostic methods. Beside the monitoring of haemodialysis efficiency, the here applied methods might be used for detection of trimethylaminuria, a genetic disorder involving a trimethylamine oxidase deficiency in which the body is unable to metabolize food-derived trimethylamine.

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Influence of catecholamines on the activation-induced tryptophan breakdown and neopterin formation in human PBMC

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Catecholamine hormones, such as adrenaline or noradrenaline, facilitate immediate physical reactions associated with a preparation for violent muscular action (Fight-or-flight response) [1]. Within the cellular immune response large quantities of interferon-γ (IFN-γ) are produced. IFN-γ stimulates enzyme GTP-cyclohydrolase I to produce neopterin in human monocyte-derived cells and 5,6,7,8-tetrahydrobiopterin (BH4) the cofactor of amino acid hydroxylase which are involved in the biosynthesis of catecholamines. Several interactions between the catecholamines and immunoregulatory pathways are well established [2]. This study investigates the effect of the catecholamines L-DOPA, dopamine, norepinephrine (noradrenaline) and epinephrine (adrenaline) on unstimulated and mitogen-stimulated peripheral blood mononuclear cells (PBMC) freshly isolated from human donations. PBMC were incubated with increasing doses of catecholamines and neopterin formation measured by ELISA (BRAHMS, Hennigsdorf, Germany) and tryptophan breakdown measured by HPLC were utilized as sensitive and convenient read-outs in supernatants collected after 48h [3]. The ratio of kynurenine to tryptophan (Kyn/Trp) was calculated as an estimate of indoleamine 2,3-dioyxgenase (IDO) activity. The stimulation of PBMC with mitogen phytohemagglutinin (PHA) increased formation of neopterin and breakdown of tryptophan. The addition of the catecholamines L-Dopa, dopamine, norepinephrine and epinephrine at concentrations up to 1000 µmol/L achieved a dose-dependent suppression of neopterin formation and tryptophan breakdown in stimulated PBMC. Whereas

L-DOPA achieved significant suppression of Kyn/Trp only at the highest concentration, the effect of dopamine, norepinephrine and epinephrine became significant already at 10-100 µmol/L. In unstimulated PBMC, a similar but smaller effect was observed. The same was true for the influence of catecholamines on neopterin production rates. Data suggest a role of catecholamines to counteract production of IFN-γ and Th1-type immune activation. Along this way of thinking, the compounds may promote Th2-type immunity because of the cross-regulatory influence which exists between the two arms of immune responses [4]. This assumption would be in line with earlier findings of catecholamines to promote an anti-inflammatory Th2-type of immunity [5].

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Coffee extracts suppress tryptophan breakdown in mitogen-stimulated peripheral blood mononuclear cells

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Coffee consumption is considered to exert influence on mood, immune system, cardiovascular disease and cancer, but the mechanisms of action by coffee and its various compounds are only partly known and understood. In this study, we investigated the immunomodulary effects of filtered extracts of coffee and decaffeinated coffee as well as caffeine on human peripheral blood mononuclear cells (PBMC) stimulated with mitogen phythaemagglutinin (PHA). The activation of PBMC was monitored by the degradation of tryptophan to kynurenine by the enzyme indoleamine 2,3-dioxygenase (IDO) and the production of the immune activation marker neopterin by GTP-cyclohydrolase I. Both these biochemical pathways are induced during cellular immune activation by the Th1-type cytokine interferon-γ (IFN-γ). Filtered extracts of coffee and decaffeinated coffee, both suppressed tryptophan degradation and neopterin formation in mitogen-stimulated PBMC efficiently and in a dose-dependent manner. For pure caffeine no relevant effect was observed. We conclude that the parallel influence of extracts on tryptophan degradation and neopterin production shows an immunosuppressive property of coffee compounds other than caffeine, which had no effect in our experiments. Most likely the various compounds that are present in coffee extracts like the antioxidant polyphenol flavaonoids caffeic acid or chlorogenic acid are more important for this effect. When extrapolating the *in vitro* results to *in vivo*, IFN-γ-mediated degradation of tryptophan could be counteracted by the consumption of coffee or decaffeinated coffee. It may increase tryptophan availability for the biosynthesis of the neurotransmitter 5-hydroxytryptamine (serotonin) and thereby improve mood and quality of life.

Dynamic interdependencies between emotional states and urinary zinc concentrations under everyday life conditions

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Zinc is a key component of many brain proteins. The main effect of zinc is N-methyl-D-aspartate (NMDA) receptor inhibition. A zinc-mediated inhibition of NMDA receptors in laboratory is usually related to learning problems and memory deficits. Clinical studies, however, consistently show that zinc levels are decreased under conditions of acute stress, depression, Morbus Alzheimer, and epilepsy. This study on a healthy 25-year-old woman tested the relation between urinary zinc levels and emotional states under real-life conditions. The proband of this study collected her entire urine for 63 days in 12 h intervals (total: 126 measurements). Urinary zinc to creatinine concentrations were measured using inductively coupled plasma optical emission spectrometry (ICP-OES). Urinary neopterin to creatinine and cortisol to creatinine concentrations were determined via HPLC and ELISA, respectively. Information on emotional states (mental energy levels, general lethargy, extraversion/introversion, well-being, irritation, anxiousness/depressiveness) in 12 h intervals was gathered through the Eigenschaftswörterliste (EWL 60 S). Time series were cross-correlated after ARIMA modelling. There was no significant ($p < 0.05$) correlation over time between urinary zinc levels and anxious/depressiveness. However, urinary zinc concentration increases preceded irritation increases with a temporal delay of 12-24 h. Moreover, urinary zinc concentration increases preceded increases in mental desactivity by 96-108, 144-156 and 156-168 h. On the other side, zinc increases in urine preceded decreases in mental activity by 156-168 h, in well-being by 108-120 h, and in extraversion by 120-132 h. There was no significant correlation between urinary zinc and neopterin concentrations, however, urinary cortisol levels were significantly positively correlated with zinc at lag 0. Although preliminary, our findings provide a first insight into the causal relations between zinc concentrations and emotional states in real life.

Biogenesis and catabolism of molybdopterin-dependent enzymes

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Abstract not received in time!

LC-MS/MS platforms for endogenous metabolites: Lessons learned from testosterone

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Liquid chromatography tandem mass spectrometry (LC-MS/MS) is a modern hyphenated analytical technology platform well established in tertiary care clinical laboratories. Major application fields encompass therapeutic drug monitoring, endocrinology, and toxicology - here LC-MS/MS has matured to an indispensable research and routine tool [1,2]. The application of LC-MS/MS in endocrinology allows the clinical chemist to replace immunological testing platforms (i.e. RIA, ELISA, EIA) with often limited performance characteristics, especially in terms of accuracy, precision and sensitivity. In addition, LC-MS/MS allows assay multiplexing, hence enabling the investigator to monitor complete metabolic pathways (i.e. in steroid analysis) with one analytical platform. However, LC-MS/MS specific technical limitations as the occurrence of "isobaric" interferences or sudden and unpredictable ion yield attenuation, better known as "ion suppression effect", have to be managed in the design phase of an assay to provide the investigator a stable analytical platform. Result traceability to international reference materials in of central importance, especially, since matrix matched calibration materials are – in contrast to drug monitoring assay – not available for endogenous analytes. The design, validation and performance evaluation of a novel online-SPE-LC-MS/MS assay will be described including the comparison to a RIA assay in routine use. Based on the lessons learned in the course of this assay introduction, prognostic remarks regarding the transfer of pteridin and tryptophan metabolites assays to LC-MS/MS technology will be discussed.

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Effects of globularifolin on cell survival, inflammation markers and free radicals

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Based on the growing interest to herbal medicine and isolates thereof, the potential effects of globularifolin, an acylated iridoid glucoside, on cell survival, inflammation markers and free radicals scavenging were investigated. Cell viability assay was conducted on human monocyte-macrophage cell line THP-1 and human peripheral blood mononuclear cells (PBMC) using the Cell-Titer Blue assay. Potential roles of globularifolin on immune system activation were evaluated by determination of tryptophan breakdown and neopterin levels in PHA-stimulated or unstimulated PBMC supernatants. In addition, influence of globularifolin on nuclear factor-κB (NF-κB) expression was quantified on THP-1Blue cells using Quanti-Blue assay. The free radicals scavenging capacity of globularifolin has been evaluated by Oxygen Radical Absorbance Capacity (ORAC) assay. Viability assay has proved that globularifolin had no toxic effect at the tested concentrations. Conversely, proportional to the dose, 7.8-1000 μM globularifolin increased cell growth of THP-1 cells by 5-65% ($p < 0.01$). Globularifolin (6.25 and 12.5 μM) also showed proliferative effect on PBMC but 12.5, 25, 100 and 200 μM globularifolin had suppressive effect on PHA stimulated cells. Compared to unstimulated cells, PHA induced tryptophan degradation and neopterin formation. However, upon addition of 50, 100 and 200 μM globularifolin, mitogen-mediated neopterin formation was dose-dependently reduced. In unstimulated THP-1Blue cells, 50-200 μM globularifolin induced a significant NF-κB expression. By contrast in LPS-stimulated cells, a significant decrease was observed in the presence of 200 μM globularifolin. A positive correlation was found between increased neopterin and NF-κB activity by globularifolin effect ($p=0.004$). Similarly, the effect of globularifolin on neopterin levels in mitogen induced cells correlated positively with and NF-κB activity in LPS stimulated cells ($p=0.001$). Relative ORAC value was found to be 0.36 ± 0.05 μmol Trolox equivalent/g which is almost three times more potent than Trolox. These results imply that globularifolin as a natural antioxidant might represent a potential immunomodulatory agent as well as proliferative agent which deserves further *in vitro* and clinical studies.

CTLA4-Ig promotes IDO mediated tolerance to murine cardiac allografts

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Costimulatory-blockade of CD28-B7-interaction with CTLA4Ig is a well-established tolerance induction strategy. Although previous in vitro studies confirm that CTLA4Ig up-regulates IDO expression in DCs, the precise mechanisms of CTLA4Ig and IDO interaction remain unclear. Here we studied if concerted immunomodulation *in vivo* by CTLA4Ig, IDO and Tregs accounts for indefinite survival of murine-cardiac-allografts. C57BL/6 IDO (WT/knock outs) mice received BALB/c hearts. Group 1[No treatment], Group 2[Donor-specific transfusion (DST)], Group 3[CTLA4Ig], Group 4[CTLA4Ig+DST], Group 5[CTLA4Ig+DST+IDO inhibitor 1-methyl-tryptophan (1-MT)] and Group 6[CTLA4-Ig+DST+αCD25mAb]. 1-MT was delivered in slow-release-pellets (at surgery or POD 50). Serum-enzyme-activity of IDO (Kyn/Trp) was analyzed by HPLC. Quantitative PCR was used for mRNA expression of IDO1/IDO2, Foxp3 and granzyme B. Anti-donor Abs were screened by FACS. Histopathology (H&E) and immunohistochemistry (for IDO, Foxp3, CD4, CD8, CD20, CD68 and C4d) of tissues was performed. Graft survival: Group 1[7.7 ± 1.9 d], Group 2[10.7 ± 1.3 d], and Group 3 [47.7 ± 29.8 d]. Group 4: Indefinite graft survival [>100 d] and tolerance without chronic rejection in IDO WT

but acute rejection [16.5 ± 5.9 d] in IDO knock out recipients. Group 5:IDO inhibition with 1-MT, either at transplant or at POD 50, abrogated CTLA4Ig+DST tolerance induction. Group 6:αCD25 mAb depletion of Tregs prevented CTLA4Ig+DST tolerance induction. Tolerant recipients had significantly higher IDO activity as compared to non-tolerant animals, which markedly correlated with intragraft IDO and Foxp3 levels on immunostaining. IDO1/IDO2 mRNA expression was similar in tolerant and non-tolerant recipients. Anti-donor-Abs were absent in all long-term-survivors. This study provides the first direct in vivo evidence that CTLA4Ig induced tolerance to murine cardiac allografts is critically dependent on synergistic cross-linked interplay of IDO and Tregs.

The GTP-metabolite guanosine plays an important role in the protection of neuronal cells under stress

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Maintenance of cell survival is crucial to neuronal function and depends on the presence of trophic and non trophic factors. Growing evidence suggests that purine nucleosides might support neuroprotection and neuroregeneration [reviewed in 1]. Effects range from induction of cell differentiation, apoptosis, mitogenesis, and morphogenetic changes, to stimulation of synthesis and/or release of cytokines and neurotrophic factors. The fact that the purine nucleoside guanosine is present in the brain under both physiological and pathological conditions, e.g. in ischemic tissue significant increases are detected [reviewed in 1], stimulated our interest to study the protective role of guanosine. It is metabolized from guanosine triphosphate (GTP) by stepwise dephosphorylation of GTP to guanosine diphosphate (GDP) and guanosine monophosphate (GMP) by ecto-nucleotidases. This pathway constitutes an alternative degradation pathway to the cleavage of GTP by the IFN-γ-inducible enzyme, GTP cyclohydrolase I (EC 3.5.4.16) [2] to 7,8-dihydroneopterin triphosphate, which is metabolized by dephosphorylation and oxidation to neopterin and 7,8-dihydroneopterin [3, 4]. Low oxygen (1%) and serum-deprivation were used as stress factors of neuronal sympathetic ganglion-like clonal rat pheochromocytoma PC12 cells. Our experiments demonstrate the remarkable potential of guanosine to rescue cells from stress-induced cell death accompanied with enhanced neurite [5, 6, reviewed in 1]. Guanosine-mediated rescue mechanisms are currently investigated, but based on earlier data, the p42/44 mitogen-activated protein kinase (MAPK) [7] and the protein kinase C-related kinase (PRK1) [8] are hypothesized as key-signaling elements. These data support current efforts to propagate purine nucleosides as neuroprotective and neuroregenerative substances.

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Rapid test for neopterin measurement in plasma or serum

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Neopterin is excreted by activated macrophages, and can be determined in serum, plasma or other body fluids. Neopterin is elevated in infections by viruses, intracellular living bacteria and parasites, in autoimmune diseases, in allograft rejection episodes, in neurological and in cardiovascular diseases as well as malignant diseases. As neopterin is usually elevated in viral infections but seldom in bacterial infections, it can be used as a basic tool for distinguishing viral infections from bacterial infections. Screening of neopterin concentrations in blood donations allows detecting acute viral infections in a non-specific way and improves safety of blood transfusions. InfectCheck® NeoPT is the first commercial available rapid test for the qualitative detection of neopterin in plasma or serum samples. The lateral flow test is intended for professional and laboratory use.

The immunochromatographic reaction is initiated by adding plasma or serum to the well of the test device. Neopterin binds to a colloidal gold-labeled monoclonal antibody on the conjugate release pad. The resulting complex flows over a nitrocellulose membrane where neopterin conjugated with a carrier protein is immobilized at the test zone. The result can be interpreted after 15 min. If the neopterin concentration in the sample is below the threshold value of 10 nmol/L, a red line at the test zone (T) can be observed. For neopterin concentration ≥10 nmol/L, the intensity of the red line at the T zone is equal to or weaker than that at the C zone or even no red line at the T zone can be observed. The intensity and speed at which the color develops depends on the neopterin concentration in the sample.

In viral lower respiratory tract infections Neopterin shows a sensitivity of 96.9% and a specificity of 54% based on a cut-off 10 nmol/L [1]. The new rapid test showed agreeable results to an ELISA in serum samples with concentrations of 4.2 to 20.3 nmol/L. The InfectCheck® NeoPT rapid test is an accurate, rapid, easy-to-use test for the detection of neopterin in plasma or serum samples. It might be especially useful in clinical settings for a rapid differentiation between bacterial and viral respiratory infections.

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Dependence of alkylglycerol monooxygenase activity on intracellular tetrahydrobiopterin concentrations

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Alkylglycerol monooxygenase is an integral membrane enzyme responsible for cleaving etherlipids, a class of lipids involved in spermatogenesis, brain structure, lens organisation and cell-to-cell signalling. Only recently the sequence for this enzyme has become

available after a more than 4 decades quest to identify it. This delay was caused primarily by the extremely high hydrophobicity of this protein and a putative di-iron centre making any purification attempt unsuccessful. In 2010 we were able to assign a sequence to this enzyme by an alternative strategy using bioinformatics to select candidate clones, expressing them heterologously in mammalian cells and measuring the enzymatic activity by a sensitive fluorescence-based HPLC assay. Alkylglycerol monooxygenase belongs to the family of tetrahydrobiopterin-dependent enzymes like the aromatic amino acid hydroxylases and the nitric oxide synthases however, dependence on intracellular cofactor concentrations has so far never been investigated. We therefore aimed to investigate the impact of a reduction of intracellular tetrahydrobiopterin on the capability of the cells to cleave etherlipids. For this we readjusted our HPLC based assay to a live cell assay, where fluorescent etherlipid substrate is directly added to the cells and the pyrenedecanoic acid product release by the cells is monitored by harvesting supernatants and analysing them by HPLC. Two different strategies were used for cofactor modulation: i) a pharmacological inhibition of the key enzyme in cofactor biosynthesis namely GTP cyclohydrolase 1 by its specific inhibitor 2,4-diamino-6-hydroxypyrimidine and ii) short hairpin RNA based knockdown of GTP cyclohydrolase 1. All experiments were performed in the murine macrophage cell line RAW264.7 which displays a high intrinsic activity of both GTP cyclohydrolase 1 and alkylglycerol monooxygenase. We could observe a significant reduction in pyrenedecanoic acid formation when cofactor levels were altered according to the two strategies indicating a high dependence of alkylglycerol monooxygenase activity on presence of sufficient cofactor levels in cultivated cells. When adding sepiapterin, a precursor of tetrahydrobiopterin, to the cells both cofactor levels and product formation could be restored to normal levels. This study shows for the first time that alkylglycerol monooxygenase is dependent on intracellular tetrahydrobiopterin levels and that its activity can be altered in intact cells by modulation of endogenous cofactor concentrations.

Physiological roles of alkylglycerols

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Alkylglycerol monooxygenase is a tetrahydrobiopterin-dependent enzyme. In addition to aromatic amino acid hydroxylases and phenylalanine hydroxylase it forms a third class of tetrahydrobiopterin dependent enzymes [1]. Alkylglycerol monooxygenase is the only enzyme known to cleave the alkylglycerol ether bond, leading to an irreversible degradation of a whole family of alkylglycerols and alkylglycerol lyso-phospholipids. Alkylglycerol lipids differ from the better studied acylglycerol lipids in the nature of the bond attaching the fatty acid side chain to the position sn1 of glycerol. This bond is an ester bond in acylglycerols, and an ether bond in alkylglycerols. When the carbon - carbon bond adjacent to this ether bond in the lipid side chain is a double bound, these compounds are called alkenylglycerols or plasmalogens. Lyso-plasmalogens are not cleaved by alkylglycerol monooxygenase but are degraded by lysoplasmalogenases. To allow the setup of hypothesis of a potential role for this enzyme in mammalian physiology, we review in our presentation what is known about physiological roles of alkylglycerols.

Alkylglycerols fed to mammals are incorporated into membranes throughout the body. Small amounts of alkylglycerols in the diet have been shown to increase the macrophage phagocytic potential in animals. Disruption of alkylglycerol biosynthesis in mice is resulted in immature sperm, disturbed brain architecture and cataract. When position 2 of alkylglycerols is attached to an ester, and position 3 to one of the common phospholipid residues, then these compounds form major membrane constituents e.g. brain and in testis and are no substrates of alkylglycerol monooxygenase. Upon cleavage of the sn2 ester bond by phospholipase A2, the resulting lyso compounds become substrates of alkylglycerol monooxygenase. Platelet activating factor (1-O-alkyl-2-acetyl-sn-phosphatidylcholine) is a highly active pleiotropic pro-inflammatory mediator, which in tissues is synthesized by an acetyltransferase from the lyso-compound released from membranes by phospholipase A2. Since this lyso-compound is also a substrate of alkylglycerol monooxygenase, this enzyme activity might have an attenuating effect on platelet activating factor biosynthesis and an augmenting effect on its degradation.

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Neopterin and prognosis of patients with gastrointestinal stromal tumors

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Increased serum or urinary concentrations of neopterin have been described in patients with tumors of different primary locations, but there are no reports on neopterin in patients with gastrointestinal stromal tumors (GIST). We have studied urinary neopterin in 46 patients with advanced/metastatic GIST. Urinary neopterin was determined by high performance liquid chromatography Urinary neopterin was increased above normal range in 19 patients with advanced/metastatic GIST. Eighteen patients have died during the observation period. The survival was not significantly different in patients with higher or lower urinary neopterin concentrations. In conclusion, urinary neopterin is increased in patients with advanced/metastatic GIST. Unlike in other advanced tumors, high urinary neopterin concentrations were not associated with poor prognosis.