ABSTRACTS OF THE JOINT INTERNATIONAL SYMPOSIA "VITAMIN D IN PREVENTION AND THERAPY" AND "BIOLOGIC EFFECTS OF LIGHT"

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Schlossberg Hotel, Homburg/Saar, Germany

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Oral presentations (OP)

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OP No. 42
PHOTOCHEMICAL INTERNALIZATION (PCI) IN CANCER THERAPY

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Photochemical internalization (PCI) is a novel technology for release of endocytosed macromolecules into the cytosol. The technology is based on the use of photosensitizers located in endocytic vesicles that upon activation by light induce rupture of the endocytic vesicles and, thereby, release macromolecules into the cytosol. PCI has been shown to enhance the biological activity of a large variety of macromolecules and other molecules that do not readily penetrate the plasma membrane, including type I ribosome-inactivating proteins (RIPs), gene-encoding plasmids, adenoviruses, oligonucleotides and the chemotherapeutic agent bleomycin. Chemotherapeutics may also be redirected to endocytic vesicles by nanocarriers or other carriers, such as cyclodextrins, and released into cytosol and activated by PCI. PCI has also been shown to enhance cross-presentation of tumor antigens in dendritic cells and, thereby, stimulate cancer vaccination. For clinical utilization, a novel photosensitizer has been developed and evaluated for PCI of bleomycin. Early phase clinical trials have shown promising results on several advanced cancers, despite the low specificity of bleomycin. Currently, a phase I/II clinical trial on cholangiocarcinoma is ongoing. An update of recent findings will be presented.

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OP No. 14
OPTIMIZING 25-OH-VITAMIN D_3 SERUM LEVELS FOR RITUXIMAB- AND OBINUTUZUMAB-MEDIATED ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY

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Background/Aim: Vitamin D_3 deficiency impairs the rituximab-mediated antibody-dependent cellular cytotoxicity (ADCC) and consequently, the outcome for elderly patients with diffuse large B-cell lymphoma (1) treated with cyclophosphamide, doxorubicin, vincristine, prednisone plus the monoclonal antibody rituximab (R-CHOP) and for patients with follicular lymphoma (2). The aim of this study was to determine the optimal 25-OH-vitamin D_3 (VD_3) level for rituximab- and obinutuzumab-mediated ADCC. *Patients and Methods:* Ten individuals (5 males, 5 females; mean age=67.7 years, range=41-79) without malignant disease or immunosuppression were included in this study after written informed consent. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation. CD16+ natural killer (NK) cells were separated by depleting all non-NK-cells magnetically. ADCC activity of NK cells was tested against CD20-expressing Daudi cells without or after anti-CD20-antibody treatment with rituximab and obinutuzumab, respectively. Cytotoxic activity was assessed by lactate dehydrogenase (LDH) release from the target cells. NK cells were studied at four different VD_3 serum levels: 1 (insufficient supply: <20 ng/ml); 2 (lower normal range=30 ng/ml); 3 (mid normal range=65 ng/ml); and 4 (high normal range=90 ng/ml). To achieve these levels, the probands were substituted with cholecalciferol. *Results:* The median VD_3 serum level before substitution was 10 ng/ml. ADCC after VD_3 substitution to level 2 (ranging from + 10% to + 147% that increased Daudi lysis, $p<0.05$). Further substitution to 65 ng/ml significantly increased ADCC activity in all 10 individuals compared to the ADCC at 30 ng/ml (ranging from + 18.4% to + 89.2% that increases Daudi lysis, $p<0.05$). Eight out of 10 individuals were further substituted to achieve 90 ng/ml. However, in 7/8 of these probands, the NK-cell-mediated ADCC was significantly reduced compared to their values at 65 ng/ml (ranging from - 23.1% to - 58.1% that decreased Daudi lysis, $p<0.05$). The extent of the substitution-induced ADCC improvement varied individually and was depended on the antibody concentration used to treat the target cells. Rituximab-mediated ADCC was significantly more affected by VD_3 levels than obinutuzumab. *Conclusion:* Our study demonstrates, for the first time, that ADCC of NK cells is optimal at median VD_3 serum levels around 65 ng/ml. Our data strongly argue for a rapid vitamin D_3 substitution before/at the beginning of R-CHOP treatment. The 65 ng/ml concentration has also been chosen as the target VD_3 level in the ongoing OPTIMAL>60 study of the German High-Grade Non-Hodgkin Lymphoma.

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3
OP No. 17
VITAMIN D AND MORTALITY

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This presentation provides an overview on the evidence of the association between serum vitamin D levels and all-cause and cause-specific mortality from large scale cohort studies, including an own meta-analysis of individual participant data of eight prospective cohort studies from Europe and the United States (1), as well as intervention studies. Particular attention is given to dose-response relationships, potential biases, causality, underlying mechanisms and implications for the design of interventions aimed to reduce mortality and their evaluation by randomized trials (2, 3).

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4
OP No. 2
EPIGENOME-WIDE VITAMIN D SIGNALING: FROM *IN VITRO* TO *IN VIVO*

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Via the metabolite 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) and its receptor, the transcription factor vitamin D receptor (VDR), vitamin D has not only a direct effect on the transcriptome of a tissue or cell type but also modulates the epigenome, *i.e.* the accessibility of chromatin for the binding of nuclear proteins, such as VDR, the pioneer factor PU.1 and the chromatin organizer CCCTC-binding factor (CTCF), to genomic DNA. In the past, vitamin D was studied preferentially in the context of calcium homeostasis and bone formation but, nowadays, the genome-wide actions of the nuclear hormone are also often monitored in cells of the hematopoietic system. This emphasizes the impact of $1,25(OH)_2D_3$ on the function of innate and adaptive immunity and a possible disease protective function of vitamin D *via* the modulation of the immune system. Next-generation sequencing technologies, such as ChIP-seq, FAIRE-seq and RNA-seq, are able to monitor of effects of VDR ligands on both the human epigenome and the transcriptome. The large number of vitamin D-modulated genomic loci supports the view that vitamin D has pleiotropic effects. Moreover, the investigation of epigenome-wide effects of vitamin D/$1,25(OH)_2D_3$ in hematopoietic *in vitro* cell culture models, such as THP-1 human monocytes, allows a direct comparison to *in vivo* experimental setups, where peripheral blood mononuclear cells (PBMCs) represent a tissue that can be isolated fast and with minimal harm for the human donor. This approach will demonstrate the significant impact of vitamin D on the epigenome both in cell culture, as well as within the human body *in vivo*.

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OP No. 25
MOLECULAR PATHOLOGY OF SKIN CANCER PHOTOCARCINOGENESIS: AN UPDATE

Marie Christine Martens, Christina Seebode, Janin Lehmann and Steffen Emmert

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Ultraviolet (UV) radiation is acknowledged to be the primary cause for photocarcinogenesis contributing to the development of skin cancer entities, such as squamous cell carcinoma (SCC), basal cell carcinoma (BCC) and melanoma. Typical DNA photoproducts and indirect DNA damage through reactive oxygen species are the results of UV radiation. UV-induced DNA damages are repaired by nucleotide excision repair, which, consequently, counteracts the development of mutations and skin carcinogenesis. Tumor suppressor genes are inactivated by mutations and growth-promoting pathways are activated so that the normal cell cycle progression is disrupted. Depending on the skin cancer entity, some genes are more often affected than others. In BCC, mutations in Patched or Smoothened are common and affect the Sonic hedgehog pathway. In SCC, *TP53* mutations

are prevalent, as well as mutations of the epidermal growth factor receptor (*EGFR*), *RAS, FYN* and *CDKN2A*. UV-induced mutations in *TP53* and *CDKN2A* are frequent in melanoma. UV-induced inflammatory processes facilitate photocarcinogenesis, including nuclear factor (NF)κB, cyclooxygenase (COX), and 6-Formylindolo[3,2-b]carbazol (FICZ). Recent studies have shown a connection between citrus consumption, alcohol consumption, hormone replacement therapy, oral contraceptives and the risk for photocarcinogenesis. Preventive measures may include, besides adequate use of sun protection and skin cancer screening at regular intervals, the oral intake of 500 mg nicotinamide (vitamin B3) twice daily.

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OP No. 34
***XERODERMA PIGMENTOSUM:*AN UPDATE**

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Nucleotide excision repair (NER) is the most versatile DNA repair system in mammals. NER can repair a variety of bulky DNA damages, including ultraviolet (UV)-induced DNA damage, thereby being essential for prevention of skin cancer. The consequences of defective NER factors are demonstrated by the three most common, but still rare, autosomal recessive NER defective syndromes: *Xeroderma pigmentosum* (XP), Cockayne syndrome (CS) and trichothiodystrophy (TTD). XP patients, who develop skin cancers already during childhood, show severe sun sensitivity, freckling and premature skin ageing in sun-exposed skin. CS patients exhibit sun sensitivity, severe neurological abnormalities and cachectic dwarfism. Clinical features of TTD patients include sun sensitivity, ichthyosis and short brittle sulfur-deficient hair. In contrast to CS and TTD patients, XP patients are prone to UV-induced skin cancers (melanoma, squamous and basal cell carcinomas). Interestingly, close to half of the XP patients with a defect in the *XPC* gene are not sun-sensitive. This proportion of patients is especially prone to skin cancer formation with early onset. In the normal population, reduced sun sensitivity is associated with a reduced skin cancer risk. Today, XP is diagnosed mainly on clinical grounds and confirmed by functional and genetic tests. At this moment, there is no causative treatment available for XP, highlighting the importance of an early diagnosis. Reduction of environmental UV exposure and regular skin cancer screenings can substantially improve prognosis. In 2016, European Reference Network Centers for XP have been established to professionalize comprehensive patient care.

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OP No. 18
VITAMIN D AND CANCER: EVIDENCE OF EPIDEMIOLOGICAL STUDIES

William B. Grant

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The ultraviolet B (UVB)-vitamin D cancer hypothesis was proposed in 1980 by the Cedric and Frank Garland brothers based on a geographical ecological study of solar radiation and colon cancer mortality rates in the United States. In the intervening 37 years, the hypothesis has been well supported by additional ecological studies, as well as observational studies, investigations of mechanisms and three randomized controlled trials. There are approximately 18 types of cancer with reduced incidence and increased survival with higher UVB exposure and/or 25-hydroxyvitamin D [25(OH)D] concentrations: bladder, breast, colon, endometrial, esophageal, gallbladder, kidney, lung, oral, ovarian, pancreatic, prostate, rectal, thyroid, vulvar cancer, Hodgkin's lymphoma, leukemia, non-Hodgkin's lymphoma and melanoma. Research has proceeded more slowly than expected due to limited respect for ecological studies and the fact that, as people try to limit solar UV exposure, geographical variations in incidence and mortality rates have decreased, problems with observational studies, such as relying on baseline 25(OH)D concentrations with follow-up periods lasting up to 20 years, and problems with randomized controlled trials often not measuring baseline and achieved 25(OH)D concentrations and not giving sufficiently high vitamin D doses. Nonetheless, the scientific evidence has reached the point supporting 25(OH)D concentrations >100 nmo/l for reducing cancer risk and increasing survival rates.

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OP No. 33
THE ECONOMIC BURDEN OF DISEASES ASSOCIATED WITH INSUFFICIENT OR EXCESSIVE UV EXPOSURE

William B. Grant

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Solar ultraviolet B (UVB) exposure is linked to good health and reduced burden of many adverse health outcomes but also increased burden of a few adverse health outcomes. Supporting evidence comes from a variety of sources, including geographic and temporal ecological studies, observational studies, clinical trials with vitamin D, as well as studies of mechanisms of vitamin D and UV exposure.

Among the recognised benefits of solar UVB exposure are proper bone metabolism, reduced risk of many types of epithelial cancer, dental caries, infectious diseases, including influenza and pneumonia, inflammatory bowel disease, multiple sclerosis, Parkinson's disease and rheumatoid arthritis, as well as adverse pregnancy and birth outcomes. Mounting evidence also supports a role of UVB exposure and vitamin D in reducing risk of autism. The list of adverse effects of solar UV exposure includes skin cancer and melanoma, as well as immunosuppression, which can increase risk of some diseases, such as cervical cancer and lymphoma. Interestingly, occupational UV exposure does not increase risk of melanoma in general. Results from published research will be used to estimate the human and economic burden of disease associated with insufficient or excessive UV exposure.

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OP No. 7
VITAMIN D AND CANCER PREVENTION IN ANIMAL MODELS

Frank R. de Gruijl

Leiden University Medical Center, Leiden, the Netherlands

In many epidemiologic studies, the risk of developing various types of cancers was found to be negatively associated with vitamin D status. The evidence for a causal relation is often considered insufficient because of lack of adequate randomized control trials on vitamin D supplementation (which is notably never, and cannot ethically be, a requirement to prove that a certain agent causes cancers in humans). However, animal studies have demonstrated the biologic plausibility of a causal relationship between vitamin D and reduced cancer development. In contrast to a strong steady growth in papers on vitamin D and cancer (from 20/year in 1980 to almost 600/year in 2015; PubMed), the growth in papers on experiments on the subject in mice or rats stagnated (at around 50/year after 1995). This indicates that mechanistic studies on the effects of vitamin D on cancer development have lagged behind. Recently, experimental studies advanced our understanding on the difference between colon and prostate cancer in interactions with calcium. Colon cancer development is impaired by calcium synergistically with vitamin D, whereas early prostate cancer development is enhanced by calcium, which enhancement is neutralized by vitamin D. This corresponds with an increase in expression of the calcium sensing receptor (CaSR) by vitamin D (1,25-dihydroxyvitamin D) in colon cancer cells (1) and a decrease in CaSR in prostate cancer cells (2). Calcium induced CaSR expression in prostate cancer cells. In epidemiology, vitamin D status shows a consistent negative association with colon cancer, whereas the association with prostate cancer is less consistent between studies. The latter could be envisaged to be at least in part due to variable dietary calcium intake. This mechanistic understanding should evidently guide future epidemiologic studies.

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OP No. 26
VITAMIN D AND UV-INDUCED SKIN CANCER IN ANIMAL MODELS

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Epidemiology shows a positive association between summer vitamin D status and the risk of skin carcinoma (squamous cell carcinoma (SCC) and basal cell carcinoma (BBC)). In contrast, experiments in mice have clearly demonstrated a negative control by vitamin D signaling (VDS) over the development of skin carcinomas. Apparently, the high summer vitamin D status is a good proxy of solar UV exposure, which is related to skin carcinoma risk, whereas the UV-induced vitamin D itself can be considered protective. In experiments using mice, VDS proved pivotal in controlling Wnt-beta catenin, Hegdehog and cMyc signaling in epidermal proliferation, differentiation and skin carcinoma development. Double knockouts of the vitamin D receptor (*Vdr*) and calcium sensing receptor on a low calcium diet developed skin tumors. Furthermore, VDS strongly potentiates repair of UV-induced DNA damage (specifically cyclobutane pyrimidine dimers) that counteracts UV-induced immunosuppression and UV-driven skin carcinogenesis. Interestingly, these effects are not necessarily caused only by the active metabolite 1,25-dihydroxyvitamin D but could as well be attributable to an alternative metabolite, 20 hydroxyvitamin D, which can be generated by CYP11A1 present in the skin. Interestingly, previously reported effects, dependent on VDR but not on 1,25 hydroxylation metabolism, might, thus, still be depended on vitamin D through 20 hydroxylation. Risk of melanoma is

related to sunburns and, if anything, it goes down with chronic sun exposure (as in people with outdoor professions). The latter will efficiently increase vitamin D status. Experiments with melanoma cell lines and mice clearly showed anti-melanoma effects of vitamin D. Hence, regular moderate sun exposure without sun burning would appear advisable to optimize vitamin D status and minimize melanoma risk.

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OP No. 1
THE D-LIGHTFUL VITAMIN D FOR HEALTH: A GLOBAL PERSPECTIVE

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Globally, 40% of children and adults are considered to be vitamin D deficient and an additional 20% insufficient. Vitamin D deficiency causes rickets in children and osteoporosis and osteomalacia in adults. It is also associated with muscle weakness and increased risk of falling. It is now recognized that almost all tissues and cells in the body have a vitamin D receptor. In addition, many cells in the body have also the capacity to convert 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D. There are a multitude of studies associating inadequate sun exposure and vitamin D deficiency with many chronic illnesses, including multiple sclerosis, rheumatoid arthritis, type 1 diabetes, cardiovascular disease, neurocognitive dysfunction, infectious diseases and many mortal cancers. Improving vitamin D status has been reported to reduce risk of type 1 diabetes, improve cardiovascular health in teenagers, reduce risk of influenza A infection in children, risk of upper respiratory tract infections in adults, type 2 diabetes, depression and neurocognitive dysfunction, cardiovascular disease, breast, prostate and colon cancer in adults. There is mounting evidence that vitamin D_3 itself might has unique biological properties that not only stabilize endothelial membranes to reduce risk of inflammation, but also influence protein synthesis to increase longevity. Various strategies are needed to improve the world's vitamin D status. These should include consideration for individual vitamin D supplementation, food fortification programs and recommendation for sensible sun exposure. For those who wish to obtain vitamin D naturally at times when sunlight cannot provide it and for those who are unable to absorbed dietary vitamin D, a novel alternative could be the use of a light-emitting diode (LED) device that is specifically tuned to emit radiation that efficiently produces vitamin D in the skin.

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OP No. 24
SENSIBLE SUNLIGHT EXPOSURE: A PRESCRIPTION FOR HEALTH

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Throughout evolution, life forms have taken advantage of the wide variety of energies that are emitted by the sun and penetrate to the earth's surface. Energies, in the visible spectrum, were utilized by plants for photosynthesis of carbohydrates. For the animal Kingdom, one of the most well-documented benefits of being exposed to energies in the ultraviolet B (UVB) spectrum (290-315 nm) is the production of vitamin D. This hormone was essential for the evolution of vertebrates once they left their ocean environment that was plentiful in calcium to the calcium deficient *terra firma*. When the skin is exposed to sunlight, ultraviolet, visible and infrared radiation penetrates into the epidermis. The longer the wavelength (lower energy), the deeper the penetration into the body. Therefore, most of the UVB radiation is absorbed in the epidermis, whereas UVA radiation is able to penetrate through the epidermis into the dermis. Visible radiation penetrates not only into the dermis but also passes through into the body cavity. There are a wide variety of sun-induced reactions that occur in the skin as a result of being exposed to sunlight. These include, among others, the production of beta endorphin, nitric oxide, carbon monoxide and adrenocorticotropin hormone. These products have a wide-ranging physiologic action in the skin, as well as systemically. Visible radiation also has an effect on the collagen elastin structure of the dermis and can affect wound healing and skin health. There are transcription factors produced by clock genes that regulate cellular circadian rhythm activity. These genes exist in most cells and can be influenced by exposure to sunlight. In the skin, expression of these genes is enhanced by exposure to UVB radiation. Little is known as to whether these genes that exist in most cells in the body are influenced by exposure to solar radiation. There needs to be recognised that non-burning sensible sun exposure is a prescription for good health.

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OP No. 29
SUNBEDS WITH UVB CAN PRODUCE PHYSIOLOGICAL LEVELS OF SERUM 25-HYDROXYVITAMIN D IN HEALTHY VOLUNTEERS

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Background/Aim: Vitamin D_3 is produced in the skin in response to ultraviolet B (UVB) irradiation. Exposure to certain UVB phototherapy and sunbeds that emit UVB increases serum 25-hydroxyvitamin D [25(OH)D] levels. Our aim was to characterize serum 25(OH)D response to regular sunbed use from several lamp outputs following their respective time exposure recommendations. *Patients and Methods:* Three groups tanned in dedicated sunbeds based on lamp outputs (100 W and 160 W low pressure fluorescent and 700 W high pressure filtered metal halide lamps) and a control group provided serum 25(OH)D samples at baseline and end-of-study. Tanning sessions occurred three times per week for the first 4 weeks as recommended by the manufacturer, based on a calculation from Health Canada to stay below the erythema levels and twice per week for the remaining 8 weeks of the study. *Results:* Mean 25(OH)D levels were increased by an average of 42 nmol/l in the sunbeds that used 100 W and 160 W fluorescents. Change in 25(OH)D was dependent on baseline 25(OH)D levels and group (p=0.003) but was not affected by age, sex, body mass index (BMI), Fitzpatrick type or length of the last tanning session. There was no significant change in 25(OH)D levels in participants using the 700 W high pressure halide lamp sunbed or in the control participants who did not use a sunbed. Skin pigmentation, ITA°, was markedly increased and skin lightness, L*, significantly decreased at the end of the 12 weeks. Both L* and ITA° were significantly correlated with 25(OH)D concentrations. *Conclusion:* Participants using standardized exposure schedules meeting Health Canada regulations in sunbeds irradiating UVB similar to summer sunshine showed continuous increases of 25(OH)D to physiological levels even after producing a tan in a controlled manner.

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OP No. 38
PARTIAL BODY UV EXPOSURE AND VITAMIN D METABOLISM IN CHRONIC KIDNEY DISEASE

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Introduction: Partial body exposure to sunlight is the usual mechanism to activate vitamin D. Especially patients with chronic kidney disease (CKD) suffer from vitamin D deficiency. In Germany (Northern latitude 48°-54°), mean serum levels of 25(OH)D in CKD patients range from 24 to 28 ng/ml. The onset of dialysis is highest in March and April, corresponding to the end of season with minor UV irradiation, and lowest in August and September. *Patients and Methods:* To imitate the natural sources of vitamin D, hemodialysis patients were exposed to partial body irradiation (front part of the legs, corresponding to approximately 15% of body surface) during their routine dialysis procedure three times weekly over a period of five months. Mean age was 61.7 years, mean duration on dialysis treatment 3.6 years. The E-D_3 irradiance of the devices was 0.33 W/m^2. *Results:* Highest increase was found for 25(OH)D after 8 weeks by 37% to 77.6 ng/ml and for 1,25$(OH)_2$D after 14 weeks by 42% to 50 pmol/l. In addition, a decrease of systolic and diastolic blood pressure (-8 and -6 mmHg, respectively) was found; the mean heart rate decreased from 84 to 80 bpm (-5%). *Conclusion:* Comparable to whole-body UV irradiation, partial body irradiation also is able to raise 25(OH)D and 1,25$(OH)_2$D to normal ranges. Moreover, as documented with whole-body UV irradiation (1) a comparable decrease of blood pressure was seen. This normalization of all vitamin D metabolites seems to be important due to the pleiotropic effects, especially in CKD and end-stage kidney disease (ESKD) patients, for improving cardiovascular comorbidities. Therefore, serial suberythemal partial body irradiation with a sun simulating UV spectrum seems to be equivalent to natural activation of the vitamin D metabolism.

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OP No. 13
VITAMIN D AND LIVER DISEASE

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Chronic liver disease (CLD) and several related extrahepatic manifestations, such as hepatic osteodystrophy, are associated with deficiency of vitamin D. In liver, vitamin D undergoes 25-hydroxylation, and 25-hydroxyvitamin D deficiency is highly prevalent in CLD patients. Vitamin D metabolite concentrations in serum are correlated with the severity of

CLD. Declining levels of carrier proteins, such as albumin and vitamin D-binding protein, might also be critical in CLD. Common variation in 25-hydroxyvitamin D metabolism is associated with liver stiffness in patients presenting with low to moderately increased elasticity. Although the susceptible genotypes confer small risks, we have speculated that the observed stiffness differences indicate an influence of 25-hydroxyvitamin D on both inflammation and fibrosis. Prospective cohort studies in patients with liver cirrhosis have reported an increased risk of mortality with low circulating 25-hydroxyvitamin D concentrations. Such patients are characterized by systemic inflammation in the setting of impaired innate and adaptive immune responses, where infectious complications are frequently the cause of death. In this review, we focus on epidemiological and functional relationships between vitamin D deficiency and CLD, followed by a discussion of the potential implications for clinical studies.

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OP No. 27
AVOIDANCE OF SUN EXPOSURE AS A RISK FACTOR FOR MAJOR CAUSES OF DEATH

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From an evolutionary perspective, there must be an evolutionary selection advantage in having adequately pigmented skin for the regional ultraviolet (UV) radiation. One possible mechanism might be differences in life expectancy; however, there is no such evidence. Based on the large prospective Melanoma in Southern Sweden (MISS) cohort (n=29,518), we assessed differences in life expectancy by sun exposure adjusted for age, income, education, marital status, smoking and comorbidity. Low sun exposure habits were found to be a major risk factor for all-cause mortality. This was caused by an increased risk of death due to cardiovascular disease (CVD) and non-cancer/non-CVD. Therefore, due to the increased life span among those with highest sun exposure, this exposure naturally results in an increased prevalence of cancer death. In addition, sun exposure increases the incidence, but is related to better prognosis of skin cancer. The findings indicate that there is a need for modification of guidelines regarding sun exposure.

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OP No. 19
CHALLENGE AND PROMISE: THE RELEVANCE OF VITAMIN D FOR PREVENTIVE MEDICINE

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Vitamin D deficiency is of high relevance for public health. While increasing evidence indicates a role of vitamin D for many extraskeletal diseases, including cancer, cardiovascular diseases or infections, the recommended dietary intake levels for the general population are mainly based on skeletal vitamin D effects. Preventing severe vitamin D deficiency by public health strategies has, therefore, the goal to erase or reduce the incidence of rickets in children and osteomalacia in adults without causing vitamin D toxicity. Assuming minimal or no sunlight exposure, the recommended dietary intake levels range from 600 to 800 IU vitamin D per day and should result in 25-hydroxyvitamin D serum concentrations of at least 50 nmol/l (20 ng/ml). There exists, however, a huge gap between these recommended dietary intake levels and the actual high prevalence of vitamin D deficiency in general populations, in particular among the elderly. Therefore, achieving the officially recommended dietary vitamin D intake levels throughout the European or American populations will require additional efforts, such as intensive food fortification or health campaigns. Data from meta-analyses of randomized controlled trials (RCTs) showing that vitamin D supplementation reduces fractures and modestly, but statistically significantly, also mortality support the role of vitamin D in preventive medicine. Additional data from large vitamin D RCTs will be available within the next few years and may change our current conclusions on the role of vitamin D for public health and for preventive medicine. At present, it seems to be reasonable to work on the implementation of the currently recommended dietary vitamin D intake levels as a promising preventive medicine strategy for the improvement of public health.

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OP No. 10
***IN SILICO* ANALYSES OF PRIMARY MELANOMA TRANSCRIPTOMIC DATA SUGGEST A PROTECTIVE ANTI-PROLIFERATIVE ROLE OF THE VITAMIN D-VDR SIGNALING AXIS *VIA* EFFECTS ON MITOCHONDRIAL TRANSLATION AND OXIDATIVE PHOSPHORYLATION GENES**

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Studies in the Leeds Melanoma Cohort (LMC), as well as others, show that lower levels of serum 25-hydroxyvitamin

$D_{2/3}$ ('vitamin D') are associated with thicker tumors, more frequent ulceration and poor prognosis. Also, vitamin D receptor (VDR) expression has a protective effect on melanoma-specific survival in the LMC (hazard ratio (HR)=0.51, $p=5.7\times10^{-8}$) and the cancer genome atlas (TCGA) melanoma data (HR=0.60, $p=0.000$). However, the molecular mechanisms underlying this protective effect are not fully understood. We have used transcriptomic (Illumina WG-DASL) and clinical data from 703 LMC melanoma primaries to identify biological processes associated with the observed protective effect. Preliminary analysis revealed that vitamin D has a VDR-dependent effect on survival: high serum vitamin D (>22 nmol/l) confers a survival benefit only in patients whose tumors express intermediate-VDR (n=465) but not in the low-VDR (n=119) and high-VDR (n=119) groups. Additionally, 776 genes were found to correlate with serum vitamin D (false discovery rate (FDR)<0.10) in the intermediate-VDR tumors but were not significant in the low-VDR tumors or high-VDR tumors, thus prompting further analyses into this group. Two independent *in silico* approaches (ReactomeFIViz and HPPiN) identified perturbations in mitochondrial translation, oxidative phosphorylation, and immune-related pathways to be associated with serum vitamin D in the intermediate VDR group. In addition, high serum vitamin D confers a significant survival benefit (HR=2.68, $p=0.009$) in patients whose tumors express high mitochondrial genes and oxidative phosphorylation genes. Thus, our study provides the first transcriptomic evidence of protective anti-proliferative effects of the vitamin D-VDR signaling axis *via* effects on mitochondrial translation and oxidative phosphorylation.

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OP No. 39
PHOTOTOXIC *VERSUS* PHOTOALLERGIC SKIN REACTIONS

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Many artificial or naturally occurring substances can act as photosensitizers. In general, photosensitizers absorb ultraviolet or visible light and, subsequently, transfer this energy to neighboring molecules. After induction by irradiation, these agents can lead to increased photosensitivity and, subsequently, to phototoxic or photoallergic skin reactions. In humans, phototoxic disorders are much more frequent than photoallergic diseases. Typically, a phototoxic dermatitis manifests with a sharply demarcated sunburn-like erythema that is confined to light-exposed body sites. Additionally, edema, vesicles and blisters may occur, depending on dosage of the photosensitizer and light intensity. Photoallergic reactions, however, resemble an allergic contact dermatitis on light-exposed skin areas. Clinically, erythema, dermal infiltration and desquamation can be observed, sometimes accompanied by papulovesicles and a quite distressing pruritus. In contrast to phototoxic skin reactions, a photoallergic dermatitis is characterized by unsharply demarcated lesions, which may, occasionally, spread. Although these two types of photoreactions can be clearly distinguished pathophysiologically, a clinical distinction can be difficult in individual cases. Therefore, an illuminated variant of the common patch test, referred to as photopatch test, was developed as a screening method to identify photosensitizers. Based on international large-scale photopatch test studies, typical reaction patterns could be deduced to define the differences between phototoxic and photoallergic skin reactions. If a putative photosensitizer does not cause a significant photopatch test reaction, other diagnostic procedures, such as, (*e.g.*) the photoprick, photoscratch or the illuminated intracutaneous test, are available. In case that the actual photosensitizer is not the test substance but a metabolite thereof, a systemic photoprovocation might be indicated.

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OP No. 9
VITAMIN D AND MELANOMA

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In 2009, we reported that higher 25-hydroxyvitamin $D_{2/3}$ levels shortly after the diagnosis of primary melanoma (Newton-Bishop, JCO 2009) were predictive of increased Breslow thickness (a potent prognostic indicator) and independently of poorer melanoma specific survival in the Leeds Melanoma Cohort. Other studies subsequently reported similar observations in the United States, Germany and Australia. Critics suggested that these observational data were confounded by associations between vitamin D levels and healthy lifestyles or that the higher vitamin D levels and better outcomes were in fact markers of less systemic inflammation. The United States study (1), however, reported evidence that vitamin D levels were predictive even after correction for C-reactive protein (CRP) levels. We confirmed that inhibition of proliferation of a proportion of melanoma cell lines can be induced by adding vitamin D to the cultures. We have also investigated other putative modes of action for vitamin D in melanoma patients, such as by moderating adverse effects of

systemic inflammation on cancer survival. We did indeed report that higher vitamin D levels were negatively associated with ulceration of primary tumors (which we have reported was associated with a "wound healing"/chronic inflammatory gene expression profile), whereas smoking was associated with ulceration (2) lending support to the view that vitamin D might be moderating inflammation in melanoma patients. If that was the case, then vitamin D might be important for immune responses to melanoma by suppressing reduced inflammation-reduced adaptive immunity. We had concerns, however, that vitamin D's immunosuppressive functions might be harmful. We, therefore, gave the practical advice that vitamin D insufficiency should be avoided by melanoma patients and that high doses of supplements should probably be avoided. We have used primary tumor transcriptomics to explore the associations between vitamin D and melanoma biology, which Sathya Muraldihar and I are going to describe in this meeting.

1 Fang S, Sui D, Wang Y, Liu H, Chiang YJ, Ross MI, Gershenwald JE, Cormier JN, Royal RE, Lucci A, Wargo J, Hu MI, Gardner JM, Reveille JD, Bassett RL, Wei Q, Amos CI and Lee JE: Association of vitamin D levels with outcome in patients with melanoma after adjustment for C-reactive protein. J Clin Oncol *34(15)*: 1741-1747, 2016.
2 Newton-Bishop JA, Davies JR, Latheef F, Randerson-Moor J, Chan M, Gascoyne J, Waseem S, Haynes S, O'Donovan C and Bishop DT: J Int Cancer *136(12)*: 2890-2899, 2015.

21
OP No. 31
SOLAR RADIATION AND MELANOMA RISK

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Cutaneous melanoma is a genetic disease as it is predominantly a cancer of pale skinned people. Genome-wide association studies have identified genetic variations in pigment genes as risk alleles and the importance of red hair variants in *MC1R* in risk, reflecting evidence of a role for burning in the sun in melanoma pathogenesis. Careful meta-analyses of case-control data performed by our group showed good evidence for reported sunburn and vacational sun exposure as risk factors within the "at risk" pale skinned populations. We reported no evidence that cumulative exposure to the sun was causal and these findings were reinforced more recently by the findings of Vuong *et al*. (1) who showed no evidence that occupational sun exposure was causal even in hot countries, such as Australia. Intermittent intense sun exposure is, therefore, identified as the exposure that is crucial in terms of melanoma risk and this is, therefore, the behavior that should be avoided in those with a history of sunburn or who have the other "at risk" phenotype of many melanocytic nevi. We have reported evidence for the determinants of serum vitamin D levels in melanoma patients and controls (2) from the UK and showed, not surprisingly, that sunny holidays are significantly associated with higher vitamin D levels. It is my view, therefore, that if people at risk of melanoma respond to public health advice to avoid intense sun exposure on holiday, it is likely that will impact on vitamin D levels attained for people living in northern Europe. It is also my view, therefore, that preventative health education advice to the pale skinned to avoid sunburn/sunbathing is important in order to reduce the rising incidence of melanoma but that the advice should be tempered with recommendation to avoid vitamin D insufficiency, especially for people living in colder climates. The fact that many studies have reported low levels of vitamin D in temperate climates suggests to me that casual exposure to the sun is unlikely to result in sufficient levels of vitamin D and levels are reported to be lower in pale skinned people. I, therefore, welcome the recommendation of the scientific advisory committee on nutrition (SACN) in the UK that all its residents need 400 IU/day by mouth.

1 Vuong K, McGeechan K, Armstrong BK; AMFS Investigators; GEM Investigators and Cust AE: Occupational sun exposure and risk of melanoma according to anatomical site. Int J Cancer *134(11)*: 2735-2741, 2014.
2 Davies JR, Chang YM, Snowden H, Chan M, Leake S, Karpavicius B, Haynes S, Kukalizch K, Randerson-Moor J, Elliott F, Barth J, Kanetsky PA, Harland M, Bishop DT, Barrett JH and Newton-Bishop JA: The determinants of serum vitamin D levels in participants in a melanoma case-control study living in a temperate climate. Cancer Causes Control *22(10)*: 1471-1482, 2011.

22
OP No. 3
THE REGULATION OF GENE EXPRESSION BY 1,25-DIHYDROXYVITAMIN D_3 IN TARGET CELLS: NEW TECHNIQUES, PRINCIPLES AND APPLICATIONS

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The hormone $1,25(OH)_2D_3$ exerts its complex biological activities in target cells through transcriptional regulation of gene networks involved in cellular differentiation and function. These regulatory activities are mediated by the vitamin D receptor (VDR) and its heterodimer partner retinoid X receptor (RXR), which bind to vitamin D response

elements (VDREs) near target genes. This binding functions to nucleate co-regulatory complexes that modify genetic and epigenetic components involved in altering gene output. Recent studies at the genome-wide level in bone, kidney and intestinal cells, as well as in cells that have undergone differentiation using ChIP-seq analysis, have revealed not only the numbers and precise sites of VDR action across these cellular genomes but also the individual properties of these collections of cistromic sites and their relationship to the genes they regulate. Two genome-wide principles of major relevance include the finding that (i) the majority of VDR binding sites are found in enhancers that are located distal to the promoters of the genes they regulate and (ii) these collections of sites are generally different not only between cells of distinct lineages but also between similar cells that are at different stages of differentiation. These VDR binding profiles account for the striking differences in the gene networks that are also seen to be regulated by $1,25(OH)_2D_3$. The former discovery supports the idea that traditional mechanistic studies of vitamin D action conducted in the past at specific genes now require re-examination. Our most recent studies have focused on ChIP-seq analyses of tissues derived from mice following treatment with $1,25(OH)_2D_3$ and other hormones, such as parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23). These analyses, coupled with clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9)-mediated enhancer deletion in mice, have allowed us to define the genomic sites of action of PTH, FGF23 and $1,25(OH)_2D_3$ at the *Cyp27b1* and *Cyp24a1* genes in the kidney and in target tissues.

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OP No. 22

VITAMIN D: CURRENT GUIDELINES AND FUTURE OUTLOOK

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Vitamin D is of public health interest because its deficiency is common and associated with musculoskeletal diseases, as well as extraskeletal diseases, such as cancer, cardiovascular diseases or infections. Several health authorities have reviewed the existing literature and published vitamin D guidelines for the general population. There was a consensus that 25-hydroxyvitamin D (25[OH]D) levels should be used to assess vitamin D status and that musculoskeletal and not extraskeletal effects of vitamin D should be the basis for vitamin D guidelines. Recommended target levels for 25(OH)D ranged from 25 to 50 nmol/l (10 to 20 ng/ml) corresponding to vitamin D intakes ranging from 10 to 20 μg (400 to 800 International Units) per day. It is of concern that significant parts of the general population do not meet these recommended vitamin D levels. In this context, it has been shown by food-based solutions for optimal vitamin D nutrition and health through the life cycle (ODIN) project that 13% of the European population has 25(OH)D levels below 30 nmol/l (12 ng/ml). This requires action from a public health perspective. It should also be stressed, however, that large vitamin D randomized controlled trials (RCTs) will be completed within the next few years and may change our understanding of the role of vitamin D in human health.

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OP No. 12

TARGETING THE CUTANEOUS VITAMIN D ENDOCRINE SYSTEM FOR PREVENTION AND THERAPY OF NON-MELANOMA SKIN CANCER

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Increasing evidence indicates a high relevance of the cutaneous vitamin D endocrine system for prevention and therapy of non-melanoma skin cancer. These emerging new roles of vitamin D are, in part, based on laboratory investigations demonstrating that (i) treatment with vitamin D compounds can reduce the number of ultraviolet (UV)-induced pyrimidine dimers and (ii) involve a crosstalk of vitamin D and P53-signaling pathways. Moreover, it has been shown that many cancer cell lines respond to the antiproliferative and pro-differentiating effects of $1,25(OH)_2D_3$, the biologically active vitamin D metabolite, *in vitro*. Consequently, $1,25(OH)_2D_3$ and analogues represent promising compounds for cancer treatment. In recent years, we have investigated expression and function of key components of the vitamin D endocrine system (VDR, CYP27A1, CYP27B1, CYP24A1) in basal cell carcinoma (BCC) and in cutaneous squamous cell carcinoma (SCC). Moreover, we have looked at the biological effects of $1,25(OH)_2D_3$ in skin cancer cell lines *in vitro*. This presentation summarizes these findings and gives an overview of our present understanding of the role of vitamin D in non-melanoma skin cancer.

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OP No. 4

VITAMIN D RECEPTOR: STRUCTURE-FUNCTION ANALYSIS

Natacha Rochel

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The Vitamin D nuclear receptor (VDR) together with its heterodimeric partner, retinoid X receptor (RXR), control various biological functions. While the isolated domains of nuclear receptors, the DNA-binding domain and the ligand-binding domain (LBD), can fulfill their prime functions of transactivation, DNA- and ligand-binding allosteric communication between the different domains is thought to be essential for the cellular function. Detailed information on the molecular mechanism of action of VDR ligands has been obtained by the elucidation of the crystal structures of the VDR-LBD complexes. Structural and functional characterization of novel VDR ligands, as well as natural metabolites, that have provided novel insights into the molecular mechanism of VDR regulation and anticancer activity will be discussed. As efficient transcription activation of VDR depends upon the interaction between the nuclear receptor and co-regulators, important details of the receptor-co-activator interaction will be presented.

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OP No. 6
TWO BROTHERS IN ARMS - NOVEL INTERACTIONS BETWEEN THE P53 TUMOR SUPPRESSOR AND VITAMIN D PATHWAYS

Daria Luschkova[1,2], Jacqueline Bellenberg[1,2], Kristina Heyne[2], Anja Pemsel[1,2], Roman Saternus[2], Thomas Vogt[2], Jörg Reichrath[2] and Klaus Roemer[1]

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Vitamin D receptor (VDR) and P53 are both transcription factors that exert important tumor suppressor functions in skin and other tissues. We have shown previously a crosstalk between these signaling pathways that involves binding of VDR to p53 and murine double minute 2 (Mdm2). Here, we report new findings that underline the relevance of this interaction. P53 comes in two flavors, carrying either a proline (P) or arginine (R) at position 72, determined by single-nucleotide polymorphism rs1042522. With respect to tumor suppression, P53-72R is a bit stronger as it is a more efficient inducer of apoptosis in response to cellular stress. P53-72P, on the other hand, is associated with increased longevity in humans for unknown reasons not related to tumor suppression and with reduced female fertility. Moreover, there is an intriguing shift in the ratio of the allele frequencies in dependence of latitude, with P53-72R being much more frequent in the North. One potential reason for this may have been recently identified: P53-72R can control fat metabolism to support obesity. In this presentation, we propose another potential evolutionary reason for the increased P53-72R frequency in the North, related to the insufficiency of vitamin D production at Northern latitudes. Through initial *in silico* studies on public databases of P53-induced genes and qRT-PCR follow-ups, we identified and confirmed the gene *CYP24A1* as significantly stronger activated in the presence of P53-72R (the Northern P53 variant) compared to P53-72P. *CYP24A1* is not a directly P53-regulated gene but is regulated by the VDR in dependence of $1,25(OH)_2D_3$. The presence of P53-72R permitted lower $1,25(OH)_2D_3$ levels to achieve similar VDR activity ($1,25(OH)_2D_3$-dependent *CYP24A1* transcript levels) as in the presence of P53-72P. The mechanisms that underlie the increased VDR activity on the *CYP24A1* gene in the presence of the P53-72R variant are currently being worked out.

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OP No. 41
PHOTODYNAMIC THERAPY OF IMMUNOSUPPRESSED PATIENTS WITH FIELD CANCERIZATION

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Patients with immunosuppression -for example after organ transplantation- have a higher risk developing actinic keratosis, Bowen's disease, superficial basal cell carcinomas and virus warts. If there is a large skin area affected by these skin lesions, therapy often proves to be very difficult. Thirty percent of the actinic keratosis in immunosuppressed patients converts into squamous cell carcinomas. Therefore, treatment before transformation is crucial. Surgical treatment options and other local therapeutic measures like the use of imiquimod (Aldara®) or diclofenac (Solaraze®) are often reaching their limits because of the expansion of the affected skin area. In these cases, photodynamic therapy (PDT) is an effective treatment method. In dermatology, the use of PDT is authorized for the indications actinic keratosis, Bowen's disease and superficial basal cell carcinomas (1). The functionality of PDT is based on the interaction of light in combination with a photosensitizer like 5-aminolevulinic acid and the oxygen in the tissue. During the photophysical reaction, toxic substances, like reactive oxygen species arise and damage the altered cells in the tissue. Possible advantages of the photodynamic therapy are a good cosmetic outcome and a low rate in side-effects. A new option is the daylight PDT with the important benefit of less pain.

1 Bédane C: Photodynamic therapy in dermatology, other indications and perspectives. Ann Dermatol Venereol *140(Suppl 2)*: 229-235 (In French).

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OP No. 32

AN UPDATE ON SOLARIUM USE AND RISK FOR MALIGNANT MELANOMA: A CRITICAL SYSTEMATIC REVIEW AND META-ANALYSIS

Barbara Burgard[1,2], Jakob Schöpe[1,3], Isabel Holzschuh[1,2], Claudia Schiekofer[1,2], Sandra Reichrath[1,2], Winfried März[4,5,6], José Ordonez-Mena[7,8,9], Stefan Pilz[10,11], Thomas Vogt[1,2], Stefan Wagenpfeil[1,3] and Joerg Reichrath[1,2]

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Background: Findings from epidemiological studies suggest an association between solarium use and primary cutaneous malignant melanoma. As, however, consensus regarding this topic is still lacking, there is an urgent reason for an updated systematic review and meta-analysis. *Materials and Methods:* A systematic literature search was conducted using MEDLINE and ISI Web of Science. Included studies were critically assessed regarding their risk of bias and methodological shortcomings. Summary risk estimates and 95% confidence intervals (CIs) were derived from random-effects meta-analyses to account for possible heterogeneity across studies. Subgroup analyses were conducted to verify the robustness of pooled results and to explore possible causes of heterogeneity. *Results:* Two cohort and twenty-nine case-control studies were eligible for systematic review and meta-analysis. Overall, the quality of included studies was poor as a result of severe limitations. Pooled results of all included observational studies suggest a weak association between ever exposure to ultraviolet (UV) radiation from a solarium and melanoma risk compared with non-exposure (odds ratio (OR)=1.19; 95% CI=1.04-1.35). Nevertheless, sensitivity analyses showed inconsistent results to some extent. *Conclusion:* Main findings indicate a weak to moderate association between exposure to UV radiation from a solarium and cutaneous malignant melanoma. However, results in subgroup analyses were inconsistent and, thus, we could not infer causation. Moreover, findings should be interpreted with caution due to several methodological shortcomings in included studies.

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OP No. 28

SHADING AND VITAMIN D-WEIGHTED EXPOSURE

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Shading in the natural and artificial environment of humans can limit the availability of solar radiation quite drastically. For the Vitamin D-weighted exposure, direct sun plays a minor role only. Instead sky radiance has been found to be the dominant factor for the solar ultraviolet (UV) exposure. To determine the vitamin D_3-weighted exposure, the solar spectral radiance must be known from all directions. The Vitamin D_3-weighted exposure itself can be calculated by integrating the incident radiance over all relevant parts of the human body that are not covered by clothing. Shading of buildings or trees reduces the incident radiance significantly. Within a city, we found reductions of typically more than 50% compared to unshaded areas. We also found reduction of exposure to be not simply proportional to the shaded portion of the sky. As expected, the actual reduction strongly depends on the location. This will be demonstrated in a movie taken by an all-sky camera. The incident radiance is also highly variable in time due to the presence of clouds. Since clouds play a crucial role in determining the actual exposure of humans, new instruments that measure sky radiance in dependence of zenith and azimuth angle in more than 100 directions simultaneously have been developed in recent years.

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OP No. 8

VITAMIN D AND HPV-INDUCED CARCINOGENESIS

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Human papillomaviruses (HPVs) infect keratinocytes of skin or mucosa and cause hyperproliferative lesions. More than 120 HPV types have been characterized. Depending on their oncogenic potential, HPV infections cause benign warts or intraepithelial neoplasia that may progress to invasive carcinoma. In particular, the genus alpha mucosal high-risk HPV types 16 and 18 have a well-established causal role in anogenital carcinogenesis, while the biology of cutaneous beta-HPVs is less well understood. Both, perturbance of the host keratinocytes by viral oncoproteins, as well as changes in the local microenvironment, critically influence the course of disease. Notably, our data provide evidence that Vitamin D affects key steps of HPV-induced carcinogenesis suggesting a beneficial role for Vitamin D in cancer prevention and/or therapy.

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OP No. 37
PHOTOTHERAPY IN DERMATOLOGY: WHERE ARE WE STANDING AND WHERE WILL WE BE GOING?

Adrian Tanew

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The introduction of oral photochemotherapy (psoralen and ultraviolet A (PUVA)), as an extremely effective treatment measure for severe plaque psoriasis in 1974, was a milestone in dermatologic therapy and has both sparked basic research on the mode of action of phototherapies, as well as promoted the development of newer phototherapeutic modalities, such as narrowband UVB (NB UVB; 311-313 nm) and UVA-1 (340-400 nm) treatment. Phototherapies can be used for a wide range of dermatological disorders due to their pleiotropic effects on various constituents of human skin. UV and PUVA down-regulate inflammatory processes and are, therefore, beneficial in the treatment of numerous inflammatory skin diseases, *e.g.*, psoriasis, eczema or lichen ruber. They also potently stimulate melanocyte proliferation, migration and activity that contribute to their therapeutic effect in vitiligo. Finally, UVA and PUVA improve sclerosing skin disorders by inducing matrix metalloproteinase expression in human dermal fibroblasts. Phototherapies have a rapid onset of action, high therapeutic efficacy and a favorable benefit/risk ratio at moderate treatment costs. The major long-term risk of phototherapies is their potential to promote skin cancer, in particular, non-melanoma skin cancer. This risk has been found to increase substantially with more than 200 exposures of PUVA therapy but appears to be much lower for narrowband UVB treatment. Data on the photocarcinogenic hazard of therapeutic doses of UVA-1 radiation are lacking. The future of dermatologic phototherapy is challenged by the advent of newer and massively promoted treatments, *e.g.* biologics and small molecules that compete for the same patients' populations. Given the high costs of these newer treatments and their as yet uncertain long-term safety, phototherapies should, however, remain a mainstay in the dermatologic armamentarium.

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OP No. 11
EXPRESSION AND MODULATION OF GENES OF PHARMACOKINETIC AND GENOME PROTECTIVE RELEVANCE WITHIN ENTERIC CELLS BY LIGANDED VITAMIN D RECEPTOR (VDR)

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In this study, we employed enteric cell models and ex vivo-based human colon explants to examine how activated vitamin D receptor (VDR) may impact upon the expression of genes relevant to metabolic disease and the bioavailability of many endogenous/xenogenous substrates. We have found that, when assessed in relation to the liganded activities of other related nuclear receptors, the expressions of *CYP2B6, CYP3A4/5* and *ABCB1* are most potently regulated through activation of VDR. Of particular note, we have established the synthetic VDR agonist EB1089 that can specifically elicit a sustained and elevated expression of *CYP3A4* mRNA and CYP3A4 enzyme, suggesting the potential for selective metabolic gene targeting through ligand design. In addition, we report members of the *UGT1A* gene family to be novel VDR regulated genes and identify a functional vitamin D response element (VDRE) within the proximal promoter region of *UGT1A1*, thus extending the known metabolic effects of vitamin D to also encompass expression of phase II (conjugating) genes. Based upon our findings, we propose that systemic vitamin D status and activating VDR ligands to be of importance for maintaining stable expression of phase II and functionally related genes that serve to provide baseline protection against the toxic effects of xeno- and endobiotic metabolites. This is an aspect of vitamin D biology of relevance to healthy aging but also could be potentially applied to modulate the pharmacokinetic/ pharmacodynamic profile of a number of co-administered drug regimes.

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OP No. 35
SOLAR UV AND OXIDATIVE STRESS: ARE ANTIOXIDANTS IN SUNSCREENS NECESSARY?

Rex M.Tyrrell

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Penetration of solar ultraviolet (UV) radiation (particularly UVA) into skin cells and tissue leads to a dramatic, diffusible and sustained oxidative stress that damages key components of skin, including structural components of the extracellular matrix of the dermis. Among the reactive oxygen species (ROS) generated by UVA radiation are singlet oxygen and hydrogen peroxide plus various radical species, including superoxide and hydroxyl radical. UVA radiation also directly leads to an immediate increase in labile iron pools as a result of ferritin degradation and cyclooxygenase-dependent release of free heme and activates superoxide generating nicotinamide adenine dinucleotide phosphate (NADPH) oxidases 1 and 4 at levels that depend on cell type. To counter these oxidizing conditions and the consequent disruption of heme and redox homeostasis, constitutive endogenous antioxidants (*e.g.* glutathione) and constitutive and inducible antioxidant enzymes have evolved in tissues, as well as inducible protective pathways. To be effective, antioxidants added to sunscreens must complement and enhance protection to a higher level than that provided by the optical filtering alone. A variety of phytochemicals (particularly flavonoids) and antioxidant vitamins are often added into optical sunscreens; however, the evidence substantiating the effectiveness of this approach in enhancing protection against acute and chronic skin damage remains limited.

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OP No. 23
CHALLENGE AND PROMISE, THE RISKS AND BENEFITS OF VITAMIN D SUPPLEMENTATION AND FORTIFICATION

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Prospective epidemiology demonstrates benefits of more sun exposure and/or vitamin D that include lower risk of osteoporosis, death, cancer, multiple sclerosis and metabolic syndrome. Most of these benefits can be achieved through vitamin D nutrition alone. However, vitamin D consumption is inadequate for most populations, because almost no food contains enough of it; food is still inadequate even in the context of the 2010 Recommended Dietary Allowance (RDA) of the Institutes of Medicine. From a public health perspective, the solution for an inadequate vitamin D nutritional supply is either to fortify more foods with more vitamin D and/or advise higher intakes of vitamin D as a dietary supplement. Both solutions face practical implementation problems: (i) Hesitation due to any potential health risk of excess vitamin D; (ii) Cost; (iii) Population-wide acceptance and adherence; (iv) Although fortification is by far the most realistic public health strategy, it faces design problems as to how to optimally target all at-risk segments of the population without risking vitamin D excess for some people. Previous risk/benefit analyses of vitamin D have focused on hypercalcemia; however, it is now accepted that to increase the risk of hypercalcemia, a long-term daily intake would be needed of vitamin D far higher than the doses relevant to public health, as based on the clinical trials of vitamin D. Putative risks about higher serum 25-hydroxyvitamin D or intake of vitamin D need to be settled and they include increased risks of falls and fractures, cancers of pancreas and prostate. To facilitate progress in the field of public health to implement greater fortification and/or supplementation with vitamin D, each of the putative health risks needs to be addressed through a proper meta-analysis.

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OP No. 21
NEW EXPERIMENTAL STRATEGIES TO IMPROVE THE ACCURACY AND PRECISION DURING QUANTITATIVE MASS SPECTRAL ANALYSIS OF VITAMIN D METABOLITES IN BIOLOGICAL SPECIMENS

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Liquid chromatography-tandem mass spectrometry (LC-MS/MS) assays have become the methods of choice for high-end analyses of vitamin D metabolites in biological samples. Unfortunately, the efficiencies of the commonly applied ionization techniques are low for vitamin D compounds as the molecules lack readily ionizable groups, thus resulting in poor detection sensitivities. In addition, lack of proper certified reference materials and severe ion suppression effects, as well as isobaric interferences from the biological matrix, further complicate and affect both precision and accuracy of the analyses. Some of these limitations can be overcome by using isotopologues of the vitamin D compounds as calibration standards. Selectivity issues,

however, remain a problem in most published LC-MS/MS assays and require more sophisticated techniques to account for these detrimental effects. The influence of method calibration, chemical derivatization and biological isomers of vitamin D compounds on specificity of analysis and their impact on systematic errors are the subject of this presentation, along with novel analytical approaches to circumvent them.

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OP No. 5
TRPV6 IS UP-REGULATED BY VITAMIN D_3 AND EXPRESSED IN SEVERAL MALIGNANCIES

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The family of transient receptor potential (TRP) channels consists mostly of non-selective cation channels with the exception of TRPV6 and TRPV5 that are highly calcium-selective channels showing an ion conductance of Ca permeability/Na permeability (PCa/PNa)>100. In the human population, two TRPV6 alleles are present that differ in 3 amino acids termed TRPV6a and TRPV6b. The Trpv6 protein of apes reflects the human TRPV6a variant, thus human TRPV6b is one of the very rare proteins that is significantly different compared to higher primates. The translational start triplet of TRPV6 is an ACG codon, which usually would be translated into threonine; however, instead, methionine is introduced into the protein sequence. TRPV6 has a restricted expression pattern in humans and is most dominantly expressed in the human placenta, exocrine pancreas and some exocrine gland tissues. In human Caco cells, a colon cancer-derived cell line, TRPV6 is up-regulated by 1,25-dihydroxyvitamin D_3. In addition, multiple binding sites for vitamin D receptor have been identified in the promoter region of *TRPV6* (1, 2). Most interestingly, TRPV6 is expressed frequently in human malignancies as prostate and breast cancer but is undetectable in the corresponding healthy tissues. From these findings it seems that TRPV6 is a promising biomarker and drug target for the treatment of several malignancies. Genetic inactivation of the *TRPV6* gene leads to hypofertility of male mice as a consequence of inadequate sperm maturation within the epididymis.

1 Wood RJ, Tchack L and Taparia S: 1,25-Dihydroxyvitamin D_3 increases the expression of the CaT1 epithelial calcium channel in the Caco-2 human intestinal cell line. BMC Physiol *1*: 11, 2001.
2 Meyer MB, Watanuki M, Kim S, Shevde NK and Pike JW: The human transient receptor potential vanilloid type 6 distal promoter contains multiple vitamin D receptor binding sites that mediate activation by 1,25-dihydroxyvitamin D_3 in intestinal cells. Mol Endocrinol *20(6)*: 1447-1461, 2006.

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OP No. 40
PDT OF ACTINIC KERATOSES AND OTHER SKIN DISEASES: AN UPDATE

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Photodynamic therapy (PTD) with methyl aminolevulinate (MAL) or aminolevulinic acid (ALA) has become a much used treatment modality for actinic keratoses, superficial basal cell carcinomas and is experimentally used to treat a variety of other skin diseases, such as acne vulgaris, necrobiosis lipoidica, as well as swimming pool granuloma. PDT is increasingly used as field therapy on large skin areas with many lesions, made possible by the introduction of daylight PDT, which uses daylight as light source. The important difference between conventional PDT and daylight PDT is the fact that illumination in daylight takes place during the formation of protoporphyrin IX, which, consequently, never accumulates. Thus, the treatment becomes painless for the patient. Inflammation, as a result of phototoxicity, is also reduced and can be further reduced by using topical glucocorticosteroids before and just after illumination. This way we can treat patients, who could not be treated previously due to pain and erythema, without compromising the efficacy of the treatment. This also opens for extended use of PDT, *e.g.* to treat children and young people, and to use PDT for new indications, such as acne and cutaneous leishmaniasis.

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OP No. 30
THE ROLE OF SKIN PIGMENTATION IN UVB INDUCED VITAMIN D FORMATION

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Vitamin D is mainly formed in skin exposed to ultraviolet B (UVB). It is typically measured in serum as the rather stable 25(OH)D. As melanin pigment in the skin absorbs UVB, it should be expected to reduce 25(OH)D formation during sun exposure. In white people, melanin is mainly situated in the lower epidermis in winter and, consequently, the influence on 25(OH)D could be minimal. Bogh *et al*. examined the UVB effect on 25(OH)D in light- and dark-skinned persons with

the same pre-treatment 25(OH)D level and found the same increase in 25(OH)D independent of their very different pigmentation (1). In a long-term study after summer, a somewhat lower increase in 25(OH)D was found in the more pigmented group. In this study, just after summer, melanin was expected to be present in the entire epidermis. However, melanin itself had very limited importance, whereas pigment genes had major influence.

1 Bogh MKB, Schmedes AV, Philipsen PA, Thieden E and Wulf HC: Vitamin D production after UVB exposure depends on baseline vitamin D and total cholesterol but not on skin pigmentation. J Invest Dermatol *130(2)*: 546-553, 2010.

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OP No. 16

HIGHLY INDIVIDUAL MAXIMUM SERUM 25(OH)D INDUCED BY UVB AND ITS SPONTANEOUS FALL IN HUMANS

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In general, there is a huge variability in 25(OH)D in most studies. The reasons for this are attributed to *e.g.* differences in ultraviolet B (UVB) exposure, latitude, season, clothing, skin pigmentation and ethnicity. By keeping these parameters constant, it was investigated what the maximal serum level became after identical UVB exposure in winter, where vitamin D intake was very low and outdoor UVB exposure not present. The maximal inter-individual serum 25(OH)D ranged from 85 to 216 nmol/l, a total difference in inter-individual increase of 131 nmol/l in maximal level (1). Following the same 22 individuals after cessation of UVB, proved the fall in 25(OH)D to be exponential with time, suggesting quantitatively larger elimination of 25(OH)D from higher levels. At high levels, the half-life of 25(OH)D was 3 months and at low levels longer than 6 months (2). This is in accordance with what might be seen in 25(OH)D decrease during winter time.

1 Datta P, Philipsen PA, Olsen P, Petersen B, Johansen P, Morling N and Wulf HC: Major inter-personal variation in the increase and maximal level of 25-hydroxy vitamin D induced by UVB. Photochem Photobiol Sci *15*: 536-545, 2016.
2 Datta P, Philipsen PA, Olsen P, Bogh M, Johansen P, Schmedes AV, Morling N and Wulf HC: Half-life in 25(OH)D after UVB exposure depends on gender and vitamin D receptor polymorphism but mainly on start level. Photochem Photobiol Sci DOI: 10.1039/C6PP00258G, 2017.

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OP No. 36

MAY NIR FINALLY BE CANCEROGENOUS? A CLOSER EXAMINATION CONSIDERING THE NEW FREE RADICAL CHARACTERISTICS FRGS AND FRTV

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Protection against or use of near infrared radiation (NIR) raise a very urgent health-related scientific question that needs to be answered under the pressure of a billion businesses. Recently, we provided experimental proof that non-ionizing NIR (700-1600 nm) under physiological irradiation doses is able to create free radicals (reactive oxygen species (ROS)/lipid oxygen species (LOS)) in *ex vivo* human skin, following Roscoe-Bunsen law and correlated to the NIR-driven increase of skin temperature. NIR was found to generate the same mixture of ROS/LOS as ultraviolet (UV) and visible light (VIS), but in smaller amounts. Application of our new concepts "Free Radical Ground State" (FRGS) and " Free Radical Threshold Value" (FRTV) to the experimental results and to literature data, allows to explain the two faces of NIR, *e.g.* the beneficial one in reducing the deleterious effects of UV by pre-irradiation with NIR, and in treatment of pathophysiological conditions, while on the opposite side, there is the damaging power of NIR expressed as erythema ab igne and their clear capability to act as cancerogenous as UVA.

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OP No. 20

VITAMIN D STATUS/SUPPLEMENTATION AND CARDIOVASCULAR DISEASE

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Experimental data demonstrate that both vitamin D deficiency and intoxication have deleterious effects on the cardiovascular system. In humans, results of cohort studies, Mendelian randomization studies and randomized controlled trials (RCTs) can be used to assess the dose-response relationship between vitamin D and cardiovascular disease (CVD) outcomes: Several cohort studies report a multivariable-adjusted non-linear increase in CVD events at circulating 25-hydroxyvitamin D (25OHD) levels <75 nmol/l. However, Mendelian randomization studies do not support these findings, indicating that the results of cohort studies may have been subject to residual confounding. Some meta-analyses of

RCTs do not rule out small beneficial vitamin D effects on surrogate parameters of CVD risk, such as arterial stiffness. The daily vitamin D doses used were equivalent to 1,000-5,333 IU. However, other meta-analyses of RCTs did not reveal a reduction in blood pressure, CVD events or CVD mortality by vitamin D supplementation. Notably, some cohort studies and a recent RCT provide evidence for adverse vitamin D effects on CVD outcomes at 25OHD levels >100 nmol/l, at least in patients with pre-existing CVD. These adverse vitamin D effects are probably mediated by a rise in plasma calcium levels, even if calcium levels remain within the reference range. In conclusion, there is currently no convincing evidence for a reduction in CVD events by vitamin D supplement use. More RCTs in individuals with deficient 25OHD levels (*i.e.* <30 nmol/l) are needed. Caution is necessary regarding long-term supplementation with vitamin D doses achieving 25OHD levels >100 nmol/l.

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OP No. 15
CLINICAL ASPECTS AND MOLECULAR DIAGNOSTICS OF SKIN AGING - ROLE OF VITAMIN D

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The effect of aging on skin functions is significant with a particular focus on skin permeability, wound healing, angiogenesis, lipogenesis, sweat production, immune function and vitamin D synthesis. With accelerating age, skin functions deteriorate due to structural and morphologic changes. Aging skin is prone to the development of several benign and malignant diseases. Because the number of persons aged 80 and older is expected to rise in the next decades, disease prevention will become an important issue. Screening examinations and prevention through public education starting at an early age regarding sun avoidance, the use of sunscreens and the importance of a balanced nutrition are the first steps for successful healthy aging. Although the fundamental mechanisms in the pathogenesis of aged skin are still poorly understood, a growing body of evidence points toward the involvement of multiple pathways. Recent data obtained by expression profiling studies and studies of progeroid syndromes illustrate that, among the most important biologic processes involved in skin aging, are alterations in DNA repair and stability, mitochondrial function, cell cycle and apoptosis, extracellular matrix, lipid synthesis, ubiquitin-induced proteolysis and cellular metabolism. Among others, a major factor that has been implicated in the initiation of aging is the physiologic decline of hormones occurring with age. Vitamin D receptor (*VDR*) gene polymorphisms have been associated with aging variables, such as hand grip strength, body mass index, blood pressure, high-density lipoprotein cholesterol, mini-mental state examination, medical history of hypertension, acute myocardial infarction, angina, venous insufficiency, dementia, chronic obstructive pulmonary disease and arthrosis in centenarians. In addition, *VDR* and *Klotho* genes maintain the molecular signaling systems that promote growth (P21), development (Wnt), antioxidation (nuclear factor erythroid 2-related factor 2/forkhead box O) and homeostasis (fibroblast growth factor 23) in tissues crucial for normal physiology, while simultaneously guarding against malignancy and degeneration. However, skin aging is not correlated with serum vitamin D levels. Only, among smokers, an inverse relationship manifests between vitamin D and plasma advanced glycated end-products-associated fluorescence. A better understanding of the molecular mechanisms of aging might open new strategies to deal with various diseases accompanying advanced age.

Posters (P)

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P No. 1
THE IMPACT OF VITAMIN D SUPPLEMENTATION ON VITAMIN D STATUS IN CHILDREN: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background: Vitamin D deficiency is a worldwide health problem in all age-groups, including children. Consequently, recommendations for vitamin D supplementation in children are important for public health and for preventive medicine. Aim: It was the aim of this systematic review and meta-analysis to investigate the impact of vitamin D supplementation on vitamin D status in children. Additionally, the half-life of $25(OH)D_3$ was estimated. *Materials and Methods:* A systematic literature search was conducted using MEDLINE and cross-referenced studies to investigate the impact of vitamin D supplementation on vitamin D status in children. Relevant parameters extracted included dosing and duration of vitamin D supplementation, as well as $25(OH)D_3$

serum level before and after supplementation. Results and *Conclusion:* In summary, our findings indicate that vitamin D supplementation is effective in raising $25(OH)D_3$ serum levels in children. Moreover, our results confirm the relevance of the baseline $25(OH)D_3$ level. The lower the baseline, the higher was the $25(OH)D_3$ increase after supplementation. Results will be presented in detail.

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P No. 2

EVALUATION OF $25OHD_3$ IN SERUM OF PATIENTS WITH BREAST CANCER AND BENIGN BREAST LESIONS

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Introduction/Aim: The antiproliferative effects of calcitriol are mediated *via* the vitamin D receptor. In previous studies, we showed that vitamin D receptor (VDR) is expressed in breast cancer and seemed to be up-regulated. The aim of this study is to evaluate whether serum levels of $25OHD_3$ in patients with breast cancer and benign breast lesions are similar or different. Low serum levels of $25OHD_3$ in patients with breast cancer could indicate a role of $25OHD_3$ in carcinogenesis of breast cancer. *Patients and Methods:* The level of $25OHD_3$ in serum was determined in patients with breast cancer matched with patients with benign breast lesions. *Results:* $25OHD_3$ level in serum was not significantly lower in patients with breast cancer compared with patients with benign breast lesions. *Conclusion:* There seems to be no clear evidence that serum levels of $25OHD_3$ play a role in carcinogenesis of breast cancer.

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P No. 3

EVALUATION OF $25OHD_3$ IN SERUM OF PATIENTS WITH VULVAR CANCER AND BENIGN VULVAR LESIONS

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Introduction/Aim: The antiproliferative effects of calcitriol are mediated *via* the vitamin D receptor. In previous studies we demonstrated that vitamin D receptor (VDR) is expressed in vulvar cancer and seemed to be up-regulated. The aim of this study is to evaluate whether serum levels of $25OHD_3$ in patients with vulvar cancer and benign vulvar lesions are similar or different. Low serum levels of $25OHD_3$ in patients with vulvar cancer could indicate a role of $25OHD_3$ in carcinogenesis of vulvar cancer. *Patients and Methods:* The level of $25OHD_3$ in serum was determined in patients with vulvar cancer matched with patients with benign vulvar lesions. *Results:* The level of $25OHD_3$ in serum was not significantly lower in patients with vulvar cancer compared with patients with benign vulvar lesions. *Conclusion:* There is no evidence that serum levels of $25OHD_3$ play a role in carcinogenesis of vulvar cancer.

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P No. 4

EXPRESSION OF VITAMIN D RECEPTOR IN SQUAMOUS EPITHELIAL VULVAR CANCER AND VULVAR INTRAEPITHELIAL NEOPLASIA

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Introduction/Aim: The antiproliferative effects of calcitriol are mediated *via* the vitamin D receptor. The aim of this study is to evaluate whether vulvar cancer expresses the vitamin D receptor (VDR) and if up-regulated when expressed compared to benign vulvar lesions. Furthermore, VDR expression in precursor lesions is examined. *Patients and Methods:* The expression of VDR in benign vulvar lesions, vulvar intraepithelial neoplasias and vulvar cancer was determined by immunohistochemistry using the Remmele score and by Western blotting. *Results:* VDR is expressed in benign vulvar lesions and vulvar cancer. Comparing benign with malignant lesions, the expression of VDR is up-regulated in vulvar cancer. Furthermore, VDR expression could be detected as cytoplasmatic, membranous and nuclear. *Conclusion:* For the first time, expression of VDR in vulvar cancer could be shown. Vulvar cancer and vulvar intraepithelial neoplasias may be a target for antiproliferative treatment with vitamin D analogues.

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P No. 5
COMBINATION OF VITAMIN D AND COX-2 INHIBITORS IN BREAST CANCER CELL LINES

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Vitamin D is known for its anticancer/anti-carcinogenic activity. Prostaglandin E2 (PGE2) is a proliferation and inflammation activating agent. The production of PGE2 is dependent on the activity of cyclooxygenase-2 (COX-2). A link between vitamin D and the metabolism of PGE2 has been shown in prostate cancer. We intended to show an impact of calcitriol as the active form of vitamin D on PGE2 metabolism. Furthermore, we were interested to explore a possible synergism of calcitriol and inhibitors of COX-2 on breast cancer. We investigated the influence of calcitriol and COX-2 inhibitors on cell growth *via* MTT test, as well as on protein expression of vitamin D and PGE2 metabolizing enzymes using Western blot immunoassays in MDA-MB-231 and MCF-7 breast cancer cell lines. The proliferation of MCF-7 cells was decreased by 10 µM calcitriol to 61% compared to untreated cells and in MDA-MB-231 to 90%. Treatment with 10 µM of the selective COX-2 inhibitor celecoxib decreased proliferation to 71% in MCF-7 and 88% in MDA-MB-231. The combined application of these substances led to a stronger growth inhibition down to 36% in MCF-7 and 72% in MDA-MB-231. COX-2 protein expression was induced in MDA-MB-231 cells after the application of 1 µM calcitriol reaching 154% and by 1 µM celecoxib to 137% and even further by their combination (208%) compared to untreated cells. The PGE2 catabolizing enzyme 15-hydroxypro-staglandin dehydrogenase (15-PGDH) could not be influenced by any of the substances. The expression of the calcitriol deactivating enzyme 24-hydroxylase was inhibited by 1 µM celecoxib in MCF-7 and MDA-MB-231 to 55% and 86% respectively compared to untreated cells, respectively. The inhibition of proliferation in the two breast cancer cell lines was demonstrated by both calcitriol and celecoxib, and was even stronger by their combination. Especially, the combined application of the two substances might be a promising therapeutic option for breast cancer patients. This result is supported by the fact that the expression of enzymes of one metabolizing pathway can be influenced by substances of the other and *vice versa*.

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P No. 6
THE IMPACT OF DOSE, BODY SURFACE AND OTHER FACTORS ON UVB-INDUCED VITAMIN D SYNTHESIS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background: Vitamin D deficiency is a worldwide health problem. Under most living conditions in Europe and North America, up to 90% of the body's requirements of vitamin D have to be fulfilled by the ultraviolet B (UVB)-induced cutaneous synthesis of this prohormone. As a consequence, it is of high scientific interest to determine the impact of various factors on UVB-induced cutaneous vitamin D production, measured as serum 25(OH)D_3 concentration. Aim: It was the aim of this systematic review and meta-analysis to investigate our present scientific knowledge on this topic. Additionally, the half-life of 25(OH)D_3 was estimated. *Materials and Methods:* A systematic literature search was conducted using MEDLINE and cross-referenced studies to investigate the impact of exposure to artificial UV-sources on vitamin D status. Relevant parameters included 25(OH)D_3 serum level before and after exposure, UV source and dose (in standard erythema dose (SED)) and time of exposure. Summary mean differences and 95% confidence intervals were derived from random-effects meta-analysis to account for possible heterogeneity across studies. Results and *Conclusion:* We found 15 papers published in the past 7 years. In summary, our study indicates that single doses between 0.75 and 3 SED result in the highest increase in serum 25(OH)D_3 per dose unit (SED). Exposure with higher single doses of UVB resulted in less pronounced increases in serum 25(OH)D_3 per dose unit. It can be concluded that UVB exposure with single doses between 0.75 and 3 SED are desirable in respect to cutaneous vitamin D synthesis. Interestingly, the increase in 25(OH)D_3 serum concentration was not proportional to the amount of exposed body surface. Partial exposure of the body surface resulted in relatively higher increase of 25(OH)D_3 serum concentration per SED (ΔH-25(OH)D/SED/% body surface) as compared to exposure of the whole body. For instance, exposure of face and hands resulted to an 8-fold higher increase in ΔH-

25(OH)D/SED/% body surface as compared to whole body irradiation. Moreover, our results confirm the relevance of the baseline 25(OH)D_3 level. The lower the baseline, the higher was the 25(OH)D_3 increase after irradiation. In the studies included in this systematic review, the half-life of 25(OH)D_3 can be estimated to be about two months.

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P No. 7

ASSOCIATION OF VITAMIN D STATUS WITH INCIDENCE, PROGNOSIS AND OUTCOME OF MALIGNANT MELANOMA: A META-ANALYSIS AND SYSTEMATIC REVIEW

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Vitamin D deficiency is associated with an increased incidence and unfavorable outcome of many malignancies; however, data on malignant melanoma are conflicting. We carried out a meta-analysis and systematic review investigating the association of vitamin D status with incidence, prognosis (*e.g.* Breslow thickness of primary tumors) and outcome (*e.g.* overall-, disease free-, as well as melanoma-specific survival) of malignant melanoma. Literature search was performed on PubMed and Web of Science until February 2017. Results will be presented.

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P No. 8

ASSOCIATION OF VITAMIN D STATUS WITH SERUM LIPID PROFILE IN PARTICIPANTS OF THE LUDWIGSHAFEN RISK AND CARDIOVASCULAR HEALTH (LURIC) STUDY

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Vitamin D deficiency is associated with bone diseases and many other health disorders. However, little is known about the relevance of vitamin D status on serum lipid profile. The goal of this large retrospective cohort study (n=3,316) was to analyze the potential association of vitamin D status with a large panel of serum parameters of lipid metabolism (cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, very low density lipoprotein (VLDL), VLDL-triglycerides, apolipoproteins (Apo) A1, A2, B, C2, C3, E) in participants of the Ludwigshafen Risk and Cardiovascular Health Study (LURIC study). Regression analysis demonstrated a strong association of 25(OH)D and 1,25(OH)2D status with HDL, Apo A1, and Apo A2 serum concentration ($p<0.001$). Additional statistical tests, including gender and age in multiple analyses, confirmed these results. Subgroup analysis showed similar results in participants with or without lipid lowering medication. Association of vitamin D status with most serum parameters of lipid metabolism was stronger in the subgroup of participants with 25(OH)D serum concentrations <30 ng/ml, while there was no or weaker association in the subgroup of participants with higher 25(OH)D serum concentrations. The overall effect of vitamin D status on serum lipid profile was rather small (low R-squared values), *i.e.* an increase of 1 ng/ml in 25(OH)D serum concentration resulted in an increase of 0.13 mg/dl in HDL serum concentration. In summary, our study supports the concept that vitamin D sufficiency exerts beneficial effects on serum lipids, reaching a plateau at 25(OH)D serum concentrations > 30 ng/ml.

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P No. 9

TANDEM AFFINITY PURIFICATION AND NANO HPLC-ESI-MS/MS REVEAL BINDING OF VITAMIN D RECEPTOR (VDR) TO P53 AND OTHER NEW INTERACTION PARTNERS IN HEK 293T CELLS

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While nuclear cofactors that contribute to vitamin D receptor (*VDR*)-mediated gene transcription, including retinoid X receptors (RXRs), nuclear co-activators (NCoAs) and co-repressors (NCoR), have been investigated extensively, little is known about cytoplasmic binding partners of VDR and the physiologic relevance of this interaction. To gain new insights into this topic, we have identified nuclear and cytoplasmic

VDR binding partners in untreated and in ultraviolet (UV)-B-exposed HEK 293T cells. Tandem affinity purification (using a pURB C Term TAP-tag) and nano high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS), that involves on-line trypsin digestion, separation of the digests by nano-HPLC and analysis by mass spectrometry using electrospray ionization, revealed binding of VDR to P53 and at least 60 other proteins (including 60 kDa heat shock protein, ribosomal proteins S2, S3, S4, S6, S22, L3, L5, L7 and nucleolin). This approach has the advantages of promoting protein denaturation in an aqueous-organic solvent (thereby reducing the derivatization of the sample and facilitating an in-depth analysis for detection and identification of proteins) and of the ability to acquire information from minimal amounts of sample. VDR-binding to P53 and to other proteins was confirmed using Western blot analysis. Previous findings demonstrated a crosstalk between P53 and VDR signaling and indicated that ribosomal proteins, including L7, are co-regulators of VDR-RXR-mediated transactivation of genes. Considering the high potential physiologic relevance of P53 and the cytoplasmic proteins that we identified as new VDR binding partners, our findings may point to previously unidentified functions and regulations of VDR that deserve systematic analysis.

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P No. 10
HUMAN PIGMENTATION, CUTANEOUS VITAMIN D SYNTHESIS AND EVOLUTION: VARIANTS OF GENES (SNPS) INVOLVED IN SKIN PIGMENTATION ARE ASSOCIATED WITH 25(OH)D SERUM CONCENTRATION

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Vitamin D deficiency is common in the Caucasian population and associated with increased incidence and unfavorable outcome of many diseases, including various types of cancer, infectious, cardiovascular and autoimmune diseases. Individual factors that predispose for a person's vitamin D status, such as skin type, have been identified but limited data exist on genetic determinants of serum 25-hydroxyvitamin D (25(OH)D) concentration. We have tested the hypothesis that variants of genes (single-nucleotide polymorphisms (SNPs)) involved in skin pigmentation are predictive of serum 25(OH)D levels. Serum 25(OH)D and SNPs (n=960) related to genes with relevance for skin pigmentation (*TYR, TYRP1, DCT, OCA2, TPCN2, SLC24A4, SLC45A2, ASIP, ATF1, MITF, POMC, PRKACB, PRKACG, PRKAR1A, PRKAR2A, PRKAR2B, TUBB3/MC1R, CDH1, CTNNB1, EDN1, EDN3, EDNRB, FGF2, KIT, KITLG, NGF, IRF4, EXOC2* and *TP53*) were analyzed in a cohort of participants of the Ludwigshafen Risk and Cardiovascular Health Study (n=2,970). A total of 46 SNPs were associated with lower or higher serum 25(OH)D levels as compared with the total cohort (median=15.5 ng/ml). We conclude that variants of genes involved in skin pigmentation are predictive of serum 25(OH)D levels in the Caucasian population. Our data indicate that out of the variants in 29 different genes analyzed, variants of 11 genes, including *EXOC2, TYR* and *TYRP1*, have the highest impact on vitamin D status. In an additional study, we analyzed if variants (SNPs, n=244) of 15 other genes (*ATP7A, DTNBP1, BLOC1S5, PLDN, PMEL, RAB27A, MYO5A, MLPH, MC1R, MITF, PAX3, SOX10, DKK1, RACK1, CNR1*) are predictive of serum 25(OH)D levels. Eleven SNPs located in 6 genes were associated ($p<0.05$) with low or high serum 25(OH)D levels, 3 out of these 11 SNPs reached the aimed significance level after correction for multiple comparisons (false discovery rate (FDR)). Our results have a fundamental importance to understand the role of sunlight, skin pigmentation and vitamin D for the human evolution.

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P No. 11
MEDICAL THESIS: COMBINING VITAMIN D SUPPLEMENTATION WITH MULTIPLE ESTABLISHED LIFE STYLE INTERVENTIONS TO OVERCOME CANCER

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Introduction: After decades and billions of dollars in research there is a growing body of evidence that the war against

cancer cannot be won by pursuing the actual strategy. The scientific background for this consideration is two-fold: (i) The genetic dogma has collapsed; (ii) Cancer appears more and more to be a metabolic disease. *Thesis:* Latest research shows that the human body is not a single being but consists of billions of human cells, bacteria, viruses and fungi, a non-linear, self-adapting system in constant exchange with the natural resources of its environment. Altering the natural environment by technical progress leads to the loss of many of these resources compromising the energy supply, the logistics and the information system of our body. We call this situation "Nature-Deficit-Effect" (NDE) (1). By providing the lost resources, well-known lifestyle factors like nutrition, physical activity, sun and vitamin D, *etc*., this effect can be overcome. However, this approach will only work if multiple adjuvant lifestyle interventions are combined and allow the evolutionary potential of the human system to realize its self-healing abilities. *Conclusion:* Evolutionary resources combined as multiple adjuvant lifestyle interventions are able to overcome NDE. As there is a growing body of evidence that cancer is a metabolic disease, even cancer should be successfully treated by this (r)evolutionary concept.

1 Spitz J and Spitz A: Cancer, Nutrition and more: The Nature-Deficit-Effect and the origin of cancer. Abstracts of the Ninth International Conference of Anticancer Research. *In*: Anticancer Res 2014, Bd. 34, S. 6181. Online verfügbar unter https://www.academia.edu/13537216/Cancer-Nutrition_and_more., zuletzt geprüft am 23.04.2017

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P No. 12

CAN WE ADAPT THE "COIMBRA-PROTOCOL" (HIGH DOSAGE VITAMIN D CURE OF MS) TO THE TREATMENT OF CANCER AS WELL?

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Introduction: Vitamin D is known to play an important role in the development and progress of cancer. In certain types of cancer, the figures of Vitamin D sensitivity go up to as much as 70 to 80%. The reason why the remaining patients do not profit from a sufficient Vitamin D level with regard to cancer is not known. *Thesis:* Due to a kind of vitamin D resistance, some patients are not able to overcome the cancer development in their body. In 2016, Carlberg and Haq suggested that the need for vitamin D supplementation depends on the vitamin D status in relation to the personal vitamin D response index of an individual rather than on the vitamin D status alone (1). In addition, Cicero G. Coimbra and his co-workers were able to produce a remission in patients with autoimmune diseases (above all multiple sclerosis (MS) but also vitiligo and psoriasis patients) by applying individual vitamin D doses up to 100,000 IU per day in a large number of cases (2). Putting this information together, it seems justifiable to make a similar approach in cancer patients. *Conclusion:* Individual high doses of Vitamin D should be tested as adjuvant therapy with regard to their efficacy not only in autoimmune disease but also in case of cancer.

1 Carlberg C and Haq A: The concept of the personal vitamin D response index. J Steroid Biochem Mol Biol (Published online 26 December 2016). http://dx.doi.org/10.1016/j.jsbmb.2016.12.011

2 Finamor DC, Sinigaglia-Coimbra R, Neves LC, Gutierrez M, Silva JJ, Torres LD, Surano F, Neto DJ, Novo NF, Juliano Y, Lopes AC and Coimbra CG: A pilot study assessing the effect of prolonged administration of high daily doses of vitamin D on the clinical course of vitiligo and psoriasis. Dermatoendocrinol *5(1)*: 222-234, 2013.

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P No. 13

PILOT STUDY OF A PUTATIVE CROSSTALK BETWEEN JAK-STAT (JANUS KINASE - SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION) AND VDR (VITAMIN D RECEPTOR) IN ALOPECIA AREATA

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The relevance of interferon-gamma (IFNγ)-mediated auto-inflammation for the pathogenesis of alopecia areata has been convincingly demonstrated. Responses to this cytokine involve JAK-STAT signaling, including activation of the latent cytosolic protein Stat1. Binding of IFNγ to its cell membrane receptor results in rapid assembly of a complete IFNγ-receptor complex with Jak 1 and Jak2. These enzymes phosphorylate one another and then phosphorylate the receptor. Receptor phosphorylation induces the formation of Stat1 docking sites. Following phosphorylation, Stat1 homodimerizes, translocates to the nucleus and binds DNA at

specific IFNγ activation sequences (GAS), where it functions either as activator or repressor of transcription. Recent findings indicate that JAK-STAT signaling may represent a promising target for prevention and treatment of alopecia areata. This emerging new concept may involve a crosstalk between JAK-STAT and vitamin D signaling whose relevance has previously been shown in several independent laboratory investigations. IFNγ-activated Stat1 can bind to the vitamin D receptor (VDR)-DNA-binding domain resulting in reduced VDR binding to vitamin D response elements (VDREs) and, subsequently, in inhibition of VDR-mediated transcription. To gain further insights into this topic, we have now analyzed immunohistochemically the expression of VDR and key components of JAK-STAT signaling in paraffin sections of alopecia areata patients. Moreover, the expression of key components of JAK-STAT signaling was analyzed in keratinocytes and skin immune cells following treatment with vitamin D compounds *in vitro*. The functional relevance of this interaction was further analyzed *in vitro* by blocking expression of key components of JAK-STAT signaling using siRNA in $1,25D_3$-treated cells. In this presentation we summarize our present knowledge concerning the crosstalk of JAK-STAT and VDR signaling in IFNγ-mediated inflammation and demonstrate first results from our pilot study concerning alopecia areata.

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P No. 14
EFFECTS OF EXTRACELLULAR CALCIUM AND 1,25 DIHYDROXY VITAMIN D_3 ON SEBORRHEA AND ACNE

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Calcium and 1,25-dihydroxyvitamin D_3 are well-known promoters of epithelial cell functions; however, their effects on the sebaceous gland and its diseases are not clearly elucidated. The *in vitro* part of our study was performed in order to evaluate extracellular calcium and 1,25-dihydroxyvitamin D_3 effects on human sebocytes. In addition, a clinical study was conducted in order to evaluate Ca^{2+} and $1,25(OH)_2D_3$ levels in acne patients and to elucidate the clinical relevance of the *in vitro* results. Morphology, ultrastructure, proliferation, lipid synthesis and apoptosis of SZ95 sebocytes were assessed *in vitro* under different concentrations of extracellular calcium (0.05-1.4 mM) with or without 1,25-dihydroxyvitamin D_3 (10^{-9} and 10^{-7} M) at 24 and 72 h in culture. Serum Ca^{2+} and $1,25(OH)_2D_3$ levels were assessed in 104 patients with acne (47 female and 57 male; 53% under the age of 25 years) by commercial assays. SZ95 sebocytes maintained at low extracellular calcium (0.05 mM) exhibited a rounded cell morphology, formed few loose colonies and tended to detach from culture plates. Numerous mitochondria, highly developed Golgi complexes and several small to large lipid droplets consistent with active cell metabolism and lipogenesis were observed. In contrast, SZ95 sebocytes maintained at high extracellular calcium (1.4 mM) were polygonal, readily expanded and formed large compact colonies firmly adherent to culture plates, whereas lipid droplets were barely detected. Increasing extracellular calcium levels significantly enhanced SZ95 sebocyte numbers and reduced lipogenesis. Reducing extracellular calcium enhanced SZ95 sebocyte caspase 3/7 activity (apoptosis) and calcium chelation by ethylene glycol tetraacetic acid resulted in enhanced lipogenesis. 1,25-dihydroxyvitamin D_3 decreased sebaceous lipogenesis as shown by functional and ultrastructural studies. The latter also detected signs of autophagy in 1,25-dihydroxyvitamin D_3-treated sebocytes. On the other hand, all patients tested exhibited serum Ca^{2+} levels inside the normal limits. In contrast, 81% of the acne patients presented at least 1,25-dihydroxyvitamin D_3 insufficiency, whereas 47% of the patients were even deficient. More young acne patients presented 1,25-dihydroxyvitamin D_3 deficiency (60%) in comparison to older ones (33%, Yates' p=0.01). In conclusion, extracellular calcium and 1,25-dihydroxyvitamin D_3 regulate sebocyte morphology and increase cell growth but decrease sebaceous lipogenesis and induce cell autophagy *in vitro*. Interestingly, the majority of acne patients presented 1,25-dihydroxyvitamin D_3 insufficiency and high rates of deficiency, especially the younger ones.

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