

EDITORIAL

NEW INSIGHTS INTO THE PATHOGENESIS OF CUTANEOUS AUTOIMMUNE DISORDERS

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T helper 17 (Th17) cells are characterized by the secretion of IL-17, a proinflammatory cytokine. They represent a newly described T helper subpopulation that is distinct from Th1 and Th2 lineages. Because of their pleiotropic activity on fibroblasts, keratinocytes, endothelial cells, neutrophils and memory T cells, Th17 cells are thought to be crucial in mediating tissue inflammation and autoimmunity. Autoimmune diseases were classically considered as Th1-mediated disorders such as rheumatoid arthritis or ‘mixed’ Th1/Th2 diseases such as inflammatory bowel diseases, systemic lupus erythematosus, bullous diseases, but new evidence suggests the deep involvement of Th17 cells in their pathogenesis that, potentially, may address a selective therapeutic approach targeting the IL23/Th17 pathway. This review summarizes the current knowledge of the pathogenic contribution of Th17 cells in select cutaneous autoimmune disorders, including lupus erythematosus, scleroderma, dermatomyositis, bullous pemphigoid and *pemphigus vulgaris*.

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DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

NEW INSIGHTS ON THE ROLE OF T CELLS IN THE PATHOGENESIS OF CELIAC DISEASE

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Despite intense investigation, the pathogenetic mechanisms leading to villous atrophy in Celiac disease (CD) remain not completely understood. The traditional interpretation is that CD4 cells recognize gliadin and develop an inflammatory reaction by production of Th1 cytokines at the mucosa level inducing CD8 cells to kill mucosal cells by a direct cytotoxic mechanism or by Fas-mediated apoptosis. Recent data, however, have shown that novel CD4 T-cells subpopulations, CD4⁺ CD25⁺ Regulatory T cells (Tregs) and Th17 cells also play a role in the ongoing inflammatory process. Both Tregs and Th17 cells are increased in active CD. However, because Tregs have a suppressive activity on inflammation, their role is controversial. In this editorial we discuss these recent findings and the hypothesis formulated to explain the increase of Tregs. To understand the pathogenesis of tissue damage of CD, we have focused on the duodenal micro-environment, introducing the new concept of “immunological niche” that in CD summarizes cellular and cytokine interactions in duodenal mucosa, where a high plasticity of T-cell subsets is present. CD is often complicated by T-cell lymphomas, especially in cases of refractory CD.

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EDITORIAL

IMMUNOHISTOCHEMICAL EXPRESSION AND LOCALIZATION OF SOMATOSTATIN RECEPTORS IN NORMAL PROSTATE, HIGH GRADE PROSTATIC INTRAEPITHELIAL NEOPLASIA AND PROSTATE CANCER AND ITS MANY FACES

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Data on the immunohistochemical expression and localization of the five somatostatin receptors (SSTRs) have been obtained by our group in separate studies concerning the many faces of prostate cancer (PCa), its precursor high grade prostatic intraepithelial neoplasia (HGPIN) and normal epithelium (Nep). This publication highlights the key findings, with special reference to: normal prostate epithelium; untreated HGPIN and PCa, both clinically and incidentally detected; PCa with NE differentiation; HGPIN and PCa following complete androgen ablation (CAA); and hormone refractory (HR) PCa. Taken together, the data obtained in these investigations demonstrate that SSTR profiling in individual patients with HGPIN and the multifaceted PCa is feasible and is of relevance to better tailor the somatostatin analogue-based treatment.

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DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

ROLE OF MAST CELLS IN INNATE AND ADAPTIVE IMMUNITY

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Mast cells play a central role in inflammatory and immediate allergic reactions and are necessary for allergic reactions. Mast cells play a role in the pathophysiology of autoimmune diseases and appear to be especially important in inflamed tissues, because they infiltrate tissues and produce a variety of cytokines. Mast cells are important for both innate and adaptive immunity in tissues that are in close contact with the environment, i.e. the skin, the airways and the lung, and the lining of the intestine. However, there are still many unsolved issues of mast cell functions, including their regulatory mechanism on cell differentiation in bone marrow; for example, the cytokines and transcription factors necessary for their differentiation and expansion, as well as the molecular mechanism underlying basophil migration from the bloodstream to peripheral tissues such as lymph nodes still need to be clarified.

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THE ANTIOXIDANT EFFECT OF FERMENTED PAPAYA PREPARATION INVOLVES IRON CHELATION

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Iron-overload is a major clinical problem in various diseases. Under this condition, serum iron which surpasses the binding capacity of transferrin is present as non-transferrin bound iron and cellular unbound Labile Iron Pool (LIP) is increased. LIP participates in the generation of free radicals, including reactive oxygen species (ROS). Increased ROS, with concomitant decrease in anti-oxidants, results in oxidative stress and toxicity to the liver, heart and other tissues, causing serious morbidity and eventually mortality. Therapeutic iron chelation reduces the LIP and thereby ameliorates oxidative stress-mediated toxicity. Many food-derived antioxidants have the capacities to scavenge ROS and chelate iron. We have reported that fermented papaya preparation (FPP) has ROS scavenging effect on blood cells in vitro or in vivo (in thalassemic patients and experimental animals). We now investigated FPP's iron chelating effect - its ability to prevent (and revert) LIP accumulation. Liver- and heart-derived cells, and RBCs were exposed to non-transferrin bound iron in the form of ferrous ammonium sulfate and the effect of FPP on their LIP content and ROS generation was measured by flow-cytometry. The results indicate that FPP reduces LIP and ROS, and suggests that its antioxidant mechanism is related, at least in part, to iron chelation.

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LOW FREQUENCY OF CD8⁺CD25⁺FOXP3^{BRIGHT} T CELLS AND FOXP3 mRNA EXPRESSION IN THE PERIPHERAL BLOOD OF ALLERGIC ASTHMA PATIENTS

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The aim of this study is to determine whether frequencies of CD8⁺CD25⁺ T cells and FoxP3 messenger RNA (mRNA) expression levels in CD8⁺ T cells isolated from peripheral blood are related to allergic asthma and disease severity. We enrolled 50 patients with allergic asthma (AA) and 25 healthy control subjects (NC) in our study. The frequencies of CD8⁺CD25⁺FoxP3^{-/+} T cells were assessed with flow cytometry, and mRNA FoxP3 level in CD8⁺ T cells was determined with real time polymerase chain reaction (RT-PCR). Asthma patients had fewer CD8⁺CD25⁺FoxP3^{bright} T cells [SA (median = 3.4%, IQR = 3.1) vs MA (median = 7.5%, IQR = 4.7)] than controls NC [median = 12.1 %, IQR = 8, P < 0.0001] but more CD8⁺CD25⁺FoxP3⁻ T cells [SA (median = 96 %, IQR = 3.1) vs MA (median = 92.5%, IQR = 4.7)] than controls NC [median = 87.9%, IQR = 9.2, P < 0.0001]. FoxP3 mRNA level was significantly decreased in CD8⁺ T cells of severe asthma patients (median = 0.82, IQR = 0.54) than that of patients with mild to moderate asthma and controls [(median = 2.29, IQR = 4.40) vs (median = 2.11, IQR = 3.2)]. The percentage of FoxP3⁺ T cells was correlated positively with the percentage of forced expiratory volume in 1 second (FEV₁) (r = 0.71, p < 0.01) in patients with severe asthma. The proportion of CD8⁺CD25⁺FoxP3^{bright} T cells and the level of FOXP3 gene expression in CD8⁺ T cells are relevant to allergic asthma and disease severity. The manipulation of FoxP3⁺CD25⁺CD8⁺ T cells may prevent chronic allergic inflammation and improve lung function during an acute allergic asthma exacerbation.

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SERUM VISFATIN IS ELEVATED IN CHINESE WOMEN WITH POLYCYSTIC OVARY SYNDROME, BUT MIGHT NOT BE A RELIABLE PREDICTOR OF THEIR GLUCOSE INTOLERANCE

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Both visfatin and polycystic ovary syndrome (PCOS) were previously reported to be in relation to abnormal glucose metabolism (AGM). The hypothesis was investigated in this paper that plasma visfatin level are elevated in Chinese women with PCOS, and could substitute oral glucose tolerance test (OGTT) as a simple predictor for their glucose intolerance. This cross-sectional study enrolled 119 women (91 newly diagnosed PCOS patients and 28 eumenorrheic age- and BMI- matched controls); anthropometric, hormonal, and metabolic parameters including serum visfatin were simultaneously measured in all participants. Plasma visfatin levels were compared between controls and PCOS subjects with various glucose metabolism status diagnosed by OGTT using 75 g of glucose. Pearson correlation coefficients were calculated to determine the correlations between various parameters. Receiver Operating Characteristic (ROC) analysis was performed to examine the diagnostic test performance of visfatin. Plasma visfatin levels were found to be significantly higher in our PCOS population compared to healthy controls ($P<0.05$). An increase in fasting visfatin concentrations with a worsening degree of glucose intolerance among PCOS patients was described. However, the difference did not reach statistical significance. In addition, visfatin was unexpectedly found to correlate with neither age, anthropometric, hormonal nor metabolic parameters. As a predictor for glucose intolerance to distinguish PCOS individuals with normal or abnormal glucose metabolism, visfatin was found to possess low potentially predictive ability according to ROC curve analysis. In conclusion, serum visfatin is significantly elevated in Chinese women with PCOS, but might not be a reliable predictor of their glucose intolerance.

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EFFECT OF TANNIN EXTRACT AGAINST *PSEUDOMONAS AERUGINOSA* PRODUCING METALLO BETA-LACTAMASE

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Carbapenems are the most potent beta-lactam agents with a broad-spectrum activity against Gram-negative and Gram-positive bacteria. They are stable in the presence of penicillinases and cephalosporinases. This study was focused on frequency of metallo beta-lactamase (MBL) among *Pseudomonas aeruginosa* strains isolated in patients with urinary tract infection, effect of tannin against PA positive strains which produced blaVIM or blaIMP and both of these genes (Species). Detection of MBL was performed by phenotypic and genotypic methods. Tannin extract was tested against *P. aeruginosa* producing MBL. During the study period, 240 *P. aeruginosa* isolates were identified. Among them 64 (26.6%) isolates were imipenem non-susceptible and confirmed by imipenem/EDTA. Our results revealed that the growth of blaVIM positive *P. aeruginosa* inhibited at 15µg/ml concentration. The experiment repeated for blaIMP-positive *P. aeruginosa* and *P. aeruginosa* which harbored blaIMP and blaVIM, the results showed 35µg/ml was the best concentration for inhibition of *P. aeruginosa*-positive blaIMP and also *P. aeruginosa* blaIMP and blaVIM. In conclusion, tannin was effective against *P. aeruginosa* producing blaVIM and blaIMP and both of them so it can be substituted with common antibiotics. The result showed significantly *P. aeruginosa*-harbored blaIMP was more responsible for imipenem resistance than *P. aeruginosa*-positive blaVIM. Interestingly, tannin was more effective against MBL – *P. aeruginosa* in comparison with current antibiotics.

PALATABILITY, DIGESTIBILITY AND EMOTIONAL PATTERN IN 60 HEALTHY VOLUNTEERS AFTER INGESTION OF AN ICED DESSERT PRESENTED IN FOUR DIFFERENT FLAVOURS: A SUBJECTIVE EVALUATION

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Several variables lead to changes in human and animal eating behavior and food choices. A pivotal role is played by food palatability, represented by food, smell, taste, texture, appearance and temperature. The aim of our study is to assess the potential differences in palatability and digestibility of four different flavoured iced desserts, consumed at the end of a standardized meal, and their impact on the emotional status of 60 healthy volunteers. Sixty healthy volunteers, after ENT and psychological assessment, were asked to fill out a Psycho-Emotional Questionnaire (PEQ) to assess their basal emotional pattern before the consumption of an iced dessert at the end of a standard meal, after which they completed an Organoleptic-Sensory Questionnaire (OSQ), a Dynamic Digestibility Questionnaire (DDQ) and again the PEQ. Four different flavors (lemon, tangerine, pineapple and chocolate) were tested on 4 consecutive days on the same subjects. Most of the 60 subjects, by means of OSQ, found taste, aspect, texture and smell of the 4 flavours pleasant, lemon and tangerine were the freshest and lightest. The DDQ identified pineapple and chocolate dessert as those less digestible. By means of PEQ we recorded an improvement in joy, mood and activation, associated with good data of digestibility and palatability after the consumption of all flavors. Our data showed that all flavors improve joy, mood and activation, after their consumption, without statistically significant differences. However, among the tested flavours, lemon and tangerine appear to be the most pleasant and those which facilitate the digestive process.

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**pPKC α -MEDIATED EFFECT ON *IN VITRO* A β PRODUCTION IN RESPONSE TO
 γ SECRETASE INHIBITOR LY411575 IN RAT CTXTNA2 ASTROCYTES**

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Alzheimer's Disease implies memory and cognitive impairment due to β amyloid accumulation, presence of reactive microglia and astrocytes, loss of synapses, neural network dysfunctions and modifications of neuronal signalling. A key role in such events is played by astrocytes, which actively secrete high levels of β amyloid protein originating from sequential cleavage of APP by α , β and γ secretases. Since inhibition of such process could represent an important strategy against the occurrence of Alzheimer's Disease, in this paper the role played by pPKC α in the *in vitro* β amyloid production in response to γ secretase inhibitor in rat cortical astrocytes is reported. pPKC α increased expression seems to be related to decreased β amyloid production in parallel to increased astrocytes viability and decreased iNOS expression in the presence of 10 μ M LY411575. Thus γ secretase inhibitor, activating pPKC α intracellular pathway could be suggested to prevent or reduce downstream toxic events, representing a useful strategy to counteract Alzheimer's disease.

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CYANIDIN REDUCES PREADIPOCYTE DIFFERENTIATION AND RELATIVE ChREBP EXPRESSION

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Adipogenesis is a continuous process even in adult adipose tissue for the presence of preadipocytes that, when subjected to appropriate stimuli can proliferate and differentiate. ChREBP, the essential transcription factor for lipogenesis, is expressed in all tissues, but mainly in lipogenic organs. In this study, we focused on ChREBP expression during preadipocytes differentiation. Since it was found that cyanidin-3 reduces body weight in mice even in the presence of a high-fat diet, by decreasing levels of blood glucose and by improving insulin sensitivity, we studied the effect of this substance on adipogenic differentiation. For this purpose we used preadipocytes obtained from subcutaneous and visceral human adipose explant tissue, characterized and stimulated to differentiate in selective media. On cytofluorimetric analysis these cells showed mesenchymal markers (CD29, CD90, CD44), whereas they were negative for hematopoietic markers (CD45, CD10, CD117, CD31). ChREBP expression levels were quantified by immunoelectron-microscopy and western blotting analysis. In this report we show that ChREBP is expressed in preadipocytes (both nuclear and cytoplasmic compartments); the cytoplasmic level of ChREBP increased by 50% on day seven of differentiation into mature adipocytes. Cyanidin reduced differentiation by 20% (as evaluated by red oil O staining) and the expression of ChREBP. In addition, cyanidin-treated cells showed abnormal morphology, a square shape with irregular size, probably due to the fact that cyanidin may interfere with the extracellular matrix. These findings suggest that dietary cyanidin, may have inhibitory effects on adipogenesis.

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REV-ERB α AND THE CLOCK GENE MACHINERY IN MOUSE PERIPHERAL TISSUES: A POSSIBLE ROLE AS A SYNCHRONIZING HINGE

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Rhythmic oscillations of cellular biological processes are driven by translational-transcriptional feedback loops that realize molecular clocks ticking in every single cell, driven by neural and humoral outputs from the suprachiasmatic nuclei of the hypothalamus that are entrained by environmental photon inputs. The nuclear receptor *REV-ERB α* has the capability to reset the molecular oscillators of peripheral tissues. The aim of our study was to evaluate the clock gene machinery function in light/dark cycles (LD) and in constant darkness (DD) exploiting in particular the *REV-ERB α* pattern of expression by using data from two independent experimental settings to reduce procedure related influences. In the LD study C57BL/6 male mice housed on a 12L:12D cycle were sacrificed at 4 h intervals. Liver, kidney, spleen, thymus and testis were harvested and blood was collected. Expression levels of *PER1*, *PER2*, *CRY1*, *CRY2*, *BMAL1*, *REV-ERB α* , *CLOCK* were evaluated by qRT-PCR. In the DD study Balb/c male mice in the third DD cycle as a continuation of the dark phase of the last LD cycle were sacrificed at 4 h intervals. Lung, heart, liver, stomach, kidney, spleen, and testis were harvested and mRNA expression of *PER1*, *PER2*, *CRY1*, *CRY2*, *BMAL1*, *REV-ERB α* , *CLOCK*, was evaluated by qRT-PCR. A statistically significant difference was found for the size of the semi-interquartile range of acrophases of clock gene expression in different organs evaluated in LD and DD conditions (4:38 \pm 1:12h versus 1:16 \pm 0:10h, $p=0.026$). A statistically significant difference was found for the acrophases of clock gene expression in different organs evaluated in LD ($p=0.01$) and in DD ($p<0.0001$). In LD study only *REV-ERB α* showed concomitant expression in the different peripheral tissues with the phase peaking around 07:03 \pm 0.8h. In

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ANALYSIS OF GENE EXPRESSION IN PENICILLIN G INDUCED PERSISTENCE OF *CHLAMYDIA PNEUMONIAE*

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Chlamydia pneumoniae is responsible for respiratory tract infections and has been associated to chronic diseases such as atherosclerosis. The involvement of *C. pneumoniae* in chronic diseases may be correlated to its ability to induce persistent forms in which *Chlamydiae* remain viable but are not cultivable. The aim of our study is to investigate *C. pneumoniae* specific gene activities associated with the development of Chlamydial persistence in a cell culture system in the presence of penicillin G. Chlamydia-infected HEp-2 cells were incubated with or without penicillin G for up to 72 hours. The relative mRNA expression levels of early and late genes in treated and untreated cell cultures were determined by Real-time RT-PCR. Our results revealed a consistent down-regulation of Chlamydial *hctA* and *hctB* genes ($p=0.012$ and $p=0.003$ respectively) in association with up-regulation of *htrA* gene ($p=0.002$) during penicillin G-induced persistence suggesting these gene sets as leading candidate for *in vivo* investigation of the development of persistent Chlamydial infection. In conclusion, the Chlamydial expression pattern of *hctA*, *hctB*, and *htrA* genes may be helpful to identify target molecules to diagnose and treat Chlamydia-associated chronic diseases.

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IS THERE A POTENTIAL APPLICATION OF A FERMENTED NUTRACEUTICAL IN ACUTE RESPIRATORY ILLNESSES? AN *IN-VIVO* PLACEBO-CONTROLLED, CROSS-OVER CLINICAL STUDY IN DIFFERENT AGE GROUPS OF HEALTHY SUBJECTS

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The role of oxidants in viral diseases is fairly complex because it includes metabolic regulation both of host metabolism and viral replication. However, a role for reactive oxygen species (ROS) and reactive nitrogen species (RNS) as mediators of virus-induced lung damage is supported by studies and antioxidants can thus be expected to act at many different levels. The aim of the present pilot study was to test an antioxidant nutraceutical approach on some relevant immunological parameters known to be affected in common seasonal respiratory tract infection. The study population consisted of 90 sedentary healthy patients, previously selected as being GSTM1-positive, divided into three groups: A) 20-40 years; B) 41-65 years; B) over 65 years. Each patients was administered a life style and dietary questionnaire. Subjects were supplemented for 6 weeks with either 9g/day (4.5g twice a day sublingually) of a fermented papaya preparation (Osato Research Institute, Gifu, Japan) or placebo. After a further month period of wash out, subjects were treated again in a crossover manner. Parameters checked were as follows: routine blood tests with WBC formula, saliva flow rate and secretory IgA and lysozyme production and redox gene expression of Phase II enzyme and SOD from upper airways cells (from nasal lavage). Salivary secretion rate showed an age-related decline and was significantly increased by FPP supplementation only in the youngest age-group ($p < 0.05$). Subjects treated with FPP showed a significantly higher lever of IgA and lisozyme production., irrespective of age group while their baseline production was significantly lower in the oldest age-group as compared to the youngest one (C vs A, $p < 0.05$). FPP treatment brought about a significant upregulation of all phase II enzyme and SOD gene expression tested in nasal lavage cells. In conclusion, FPP supplementation during 1 month resulted in higher salivary IgA and increase in phase II and SOD enzyme expression, i.e the most important antioxidant in the respiratory tract. The biological significance of these effects i.e., whether it will help reducing the whole respiratory oxidative stress in the human airway and, hopefully, the incidence and/or severity of URTI remains to be demonstrated in longer clinical trials.

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DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

EFFECTS OF VISFATIN/PBEF/NAMPT ON FEEDING BEHAVIOR AND HYPOTHALAMIC NEUROMODULATORS IN THE RAT

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Visfatin, also known as pre-B cell colony enhancing factor (PBEF) or nicotinamide phosphoribosyltransferase (NAMPT), is a cytokine that is produced by adipose tissue, skeletal muscle, liver and immune cells. We studied the effects of visfatin/PBEF/NAMPT on feeding behavior, hypothalamic steady state concentrations of aminergic neurotransmitters and hypothalamic mRNA levels of anorexigenic peptides, such as cocaine- and amphetamine-regulated transcript (CART) peptide, corticotropin-releasing hormone (CRH), proopiomelanocortin (POMC), and orexigenic peptides, such as agouti-related peptide (AgRP) and neuropeptide Y (NPY). Forty-eight rats were injected in the arcuate nucleus (ARC) of the hypothalamus with either saline or visfatin/PBEF/NAMPT (3 µg). Food intake was recorded 1, 2 and 24 h following injection, and either dopamine (DA), norepinephrine (NE), serotonin (5-hydroxytryptamine, 5-HT) or peptide gene expression were evaluated 2 and 24 h after visfatin/PBEF/NAMPT administration. Compared to vehicle, visfatin/PBEF/NAMPT significantly increased food intake, as evaluated 1, 2 and 24 h post-injection. Visfatin/PBEF/NAMPT treatment led to a significant decrease of DA steady state concentration, CART and CRH mRNA levels. Consequently, visfatin/PBEF/NAMPT could play an orexigenic role in the ARC, and the effect could be mediated by modulation of DA, CART and CRH activity in the hypothalamus.

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CLOCK GENE EXPRESSION IN MOUSE KIDNEY AND TESTIS: ANALYSIS OF PERIODICAL AND DYNAMICAL PATTERNS

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Molecular clocks drive circadian rhythmicity of cellular functions in peripheral tissues and organs, kidney included, whereas in the testis this clockwork seems constitutively active. We have evaluated the periodicity and the dynamics of expression of the clock genes *BMAL1*, *CLOCK*, *PER1*, *PER2*, *CRY1*, *CRY2* and *REV ERB α* over 24 h in the kidney and testis using a mouse model. The periodicity was explored by single cosinor, and dynamics were explored by calculation of fractional variations of gene expression related to time intervals. Kidney and testis were harvested at 4-h intervals over a 24-h period from eight-week-old C57BL/6 male mice housed individually on a 12 h light (L)-dark (D) cycle (lights on at 08:00 h; lights off at 20:00 h) and mRNA was extracted and analyzed by Quantitative Real-time Reverse Transcription PCR. A statistically significant difference was evidenced between kidney and testis for the original values of expression level of *BMAL1*, *PER1*, *PER2*, *CRY1*, *CRY2* and *REV ERB α* . A statistically significant difference was evidenced between kidney and testis for the fractional variation of *BMAL1*, *PER2*, *CRY1*, *CRY2* and *REV ERB α* . A significant 24-h rhythmic component was found for *BMAL1*, *CLOCK*, *PER1*, *PER2*, *CRY1*, *CRY2* and *REV ERB α* in the kidney, whereas no core clock gene showed circadian rhythmicity in the testis. Fractional variations provided significant circadian rhythms for *BMAL1*, *PER2*, *CRY1*, *CRY2* and *REV ERB α* in the kidney, whereas in the testis the fractional variation calculations showed no circadian rhythmicity, but quantitative comparison showed statistically significant differences in only 16.7% of the time points studied. In conclusion, in the kidney the clock gene machinery shows circadian oscillation of mRNA levels and time-related variations in the rate of change of clock gene expression. In the testis the clock genes do not show circadian rhythmicity of expression and the dynamics of variation are not characterized by a periodical pattern, but are quantitatively similar to those observed in the kidney. These data suggest that in the testis the clock gene machinery shows a tissue-specific pattern of function and clock genes may play a different role in the

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