Session 5. Lipids: Metabolism and Biological Functions

Lectures

L5.1

Dolichol metabolism as key regulator of protein glycosylation

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Background: The Congenital Disorders of Glycosylation form a group of human genetic diseases with abnormal protein glycosylation. Protein N-glycosylation is initiated in the Endoplasmic Reticulum by glycosylation of the lipid dolichol. Genetic defects in this process lead to a (partial) lack of complete N-glycans on proteins and a multisystem disease with neurological symptoms, abnormal coagulation, endocrine abnormalities, etc. However, in a subgroup of patients, we found surprising clinical presentations such as isolated heart disease, isolated muscle dystrophy or dominant visual loss and ichthyosis. We set out to perform biochemical and genetic investigations to unravel the gene defects in these patients and to start understanding these clinical specificities.
Results: *Via* a combined genetic and biochemical approach, we could identify mutations in dolichol cycle genes in all patients with specific clinical symptoms. These included the most upstream defect in steroid 5a-reductase 3 (*SRD5A3* [1]), dolichol kinase (*DOLK* [2]), and the dolichol-P-mannose synthase genes *DPM2* [3] and *DPM3* [4]. Complementation in yeast mutants confirmed the functional relevance of *SRD5A3* and *DOLK* mutations. For SRD5A3, presenting with visual loss, mass spectrometric analysis of polyprenoids revealed the presence or even accumulation of dolichols in yeast mutants and patient plasma. The presence of residual dolichol irrespective of absent SRD5A3 protein could suggest the presence of an alternative pathway for dolichol synthesis. DOLK deficient patients presented with isolated cardiomyopathy. Analysis of patient transplanted heart showed that deficient O-mannosylation, which is also dependent on dolichol-P-mannose availability, was the underlying cause of heart disease. Finally, defects in dolichol-P-mannose synthesis resulted in muscle dystrophy, due to defective O-mannosylation in muscle, as observed in patient muscle biopsies.
In conclusion, our results reveal that biochemical regulatory effects in dolichol metabolism, rather than differential gene expression levels, determine the diverse clinical outcomes in genetic disorders of the dolichol cycle.

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L5.2

The role of fatty acid synthase in cell metabolism and cancer progression

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Fatty acid synthase (FASN) is a key enzyme of *de novo* fatty acid synthesis. The main role of FASN is synthesis of fatty acids constituting the major energy depot in organism, that occurs mainly in liver and adipose tissue. The role of mammary gland FASN in providing suitable lipid content in milk during lactation is also widely described. Recent research, also with the use of animal FASN knockout models, provided new information about other functions of this enzyme in different tissues. These include: synthesis of precursor of PPARα natural ligand in liver; the role in insulin dependent regulation of nitric oxide synthase in epithelium; cardioprotective role in heart while proatherogenic role in macrophages; contribution in the control of food intake in hypothalamus, maintaining endoplasmic reticulum function in liver.
Among the latest research concerning FASN, definitely most of articles refer to the role of this enzyme in cancer development and progression. Fatty acid synthesis plays a key role in quickly proliferating cancer cells as fatty acids are the building blocks for formation of cell membrane phospholipids, signaling molecules (i.e. phosphatidylinositol, diacylglycerol) and a substrate for protein modification (i.e. palmitoilation). Moreover, fatty acids are component of lipid rafts that play an important role in health and disease including carcinogenesis. Two decades ago FASN was identified as an oncoantigen 519 (OA 519) in breast cancer with poor prognosis. FASN activity/gene expression is elevated in various types of cancer. Moreover, high expression of FASN in cancer cells is associated with poor prognosis. The overexpression of FASN gene in cancer cells is probably caused by an action of products of mutated suppressor proteins genes (i.e. p53) and oncogenes (i.e. Akt). The level of FASN protein is also elevated in serum of cancer patients, thus the serum level of this enzyme could be potentially added to the panel of cancer markers. A number of studies, mainly performed *iv vitro* in cancer cell lines, revealed the cytostatic and cytotoxic effects of FASN inhibitors in many tumor cells. These effects are associated with cell growth arrest and induction of apoptosis. The FASN inhibitors belongs to natural compounds, like epigallocatechin gallate present in green tea or plant derived flavonoids like luteolin, or are the synthetic FASN inhibitors (i.e. C75, C93). In conclusion recent research suggests an important role of FASN in development, progression and diagnosis of cancer. Moreover, FASN inhibitors are considered as a new selective therapeutic approach in cancer therapy.

L5.3

Oxysterols — from lipid peroxidation products to signaling molecules

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Oxysterols are oxidized cholesterol derivatives containing additional hydroxyl (-OH) or keto (=O) groups attached to the cholesterol backbone. They originate by either reactive oxygen species (ROS)-mediated cholesterol oxidation or enzymatic reactions. The most important enzymatically formed endogenous oxysterols are 24(S)-hydroxy-, 25-hydroxy- and 27-hydroxycholesterol. 24(S)-hydroxycholesterol (24-(S)HC) is the most abundant oxysterol in the brain. Conversion of cholesterol to 24-(S)HC by CYP46A1 is important for cholesterol removal from the brain. 25-HC is synthesized by microsomal non-heme iron-containing cholesterol 25-hydroxylase and is sulfated by sulfotransferase SULT2B1b to 25-HC 3-sulfate (25-HC3S) which decreases intracellular lipid biosynthesis. 27-HC is synthesized by mitochondrial CYP27 and may further be converted to the respective carboxylic acid (cholestenoic acid) by the same enzyme. Conversion of cholesterol to 27-HC and cholestenoic acid is important for cholesterol removal from cells which have no direct contact with plasma high-density lipoproteins (HDL), and CYP27 deficiency results in the accumulation of cholesterol in the brain and tendons (cerebrotendinous xanthomatosis). All these oxysterols as well as CY3A4-generated 4β-cholesterol are potent agonists of liver X receptors (LXR) and sterol regulatory element-binding proteins (SREBPs), and regulate cholesterol synthesis, uptake from plasma lipoprotein and export from the cells to HDL by activating these two transcription factors. Various ROS-dependent cholesterol oxidation products such as 7-keto, 7α-hydroxy-, 7β-hydroxy- or 5,6-epoxy-cholesterol are formed in vivo under oxidative stress conditions or are provided from alimentary sources where they originate during food preparation and storage. These oxysterols may induce apoptosis, endoplasmic reticulum stress, impair endothelium-dependent vasorelaxation and oxidation of plasma lipoproteins. Under physiological conditions total plasma oxysterols concentration is within low micromolar range, and 27-HC and 24(S)-HC are the most abundant ones. In various diseases associated with oxidative stress total oxysterols may increase to 20–30 μM which is accounted for by ROS-dependent cholesterol oxidation products.

Oral presentations

O5.1

The potential role of polyisoprenoids in *Arabidopsis thaliana* response to heat treatment

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Polyisoprenoids, the linear polymers of isoprenoid units, are present in all living organisms and function in multiple cellular processes including GPI anchor biosynthesis, protein glycosylation and modulation of the properties of biological membranes. In plants, enzymes responsible for polyisoprenoid biosynthesis, the cis-prenyltransferases (CPTs), have been implicated in response to unfolded proteins, abscisic acid (ABA) treatment, sugar availability and adaptation to adverse environmental factors, such as cold, drought and osmotic stresses. *Arabidopsis* genome encodes ten CPTs, of which nine show homology to yeast enzymes. So far, only three *Arabidopsis* CPTs have been partially characterized (CPT1, CPT6 and LEW1).
Examination of *Arabidopsis* transcriptome after heat treatment, using microarray technology, revealed a significant upregulation of *CPT7* transcript upon exposure to high temperature, while none of the remaining CPTs showed response to heat stress. Additionally, *CPT7* transcript upregulation was further enhanced in heat hypersensitive *hd2c-3* plants, carrying a null mutation in HD2C histone deacetylase, suggesting that HD2C in a negative regulator of *CPT7* expression during heat stress exposure. When exposed to high temperature, *cpt7* null mutant plants displayed a heat hypersensitive phenotype. Therefore, we hypothesize that lipid products of CPT7 might be involved in plant acclimation to high temperature conditions. The analysis of lipid profile of *cpt7* and *hd2c-3* plants after heat treatment is currently conducted.

O5.2

The impairment of palmitate-derived autophagy depends on stearoyl-CoA desaturase 1 enzymatic activity

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Obesity-associated insulin resistance and impaired β-cell function are key hallmarks of type 2 diabetes (T2D) occurrence. Conclusively, the mechanisms by which sustained elevated levels of free saturated or unsaturated fatty acids (FA) coordinate progression of β-cell failure and poor survival in T2D species remain not fully elucidated.
The reciprocal communication between apoptosis, autophagy pathway and intracellular lipids has been abundantly implicated in recent studies. Additionally, autophagy is necessary to maintain proper architecture and undisturbed functioning of pancreatic β-cells. Latest findings suggest that obese diabetic subjects and β-cells exposed to FA develop an impairment of autophagic turnover. Considering the stearoyl-CoA desaturase 1 (SCD1) is a critical lipid metabolism enzyme that regulates cellular ratios of saturated/monounsaturated FA, and it demonstrates a protective action in β-cells against lipotoxicity, its engagement in autophagy awaits further attention.
The aim of the present study was to investigate the role of SCD1 activity in the palmitate-induced autophagy within INS-1E cell line which was used as the pancreatic β-cell model for *in vitro* analyses. Augmented level of apoptotic markers (cleaved caspase 3, PARP) was observed in INS-1E cells treated with palmitate and specific SCD1 inhibitor whereas accumulation of autophagic marker (LC3BII) was noticeably alleviated in comparison to control. Furthermore, additional treatment of the cells with monensin, an inhibitor of autophagy at the step of fusion, potentiated apoptosis and suggests SCD1 activity is necessary for flawless autophagic turnover. Ablation of SCD1 activity affected accumulation of distinct phospholipid classes such as cardiolipin, phosphatidylethanolamine and phosphatidylinositol, the primary mediators of the autophagic/apoptotic sequelae and fundamental constituents of cellular membranes. Moreover, co-supplementation of palmitate with the SCD1 inhibitor led to changes in a saturation status of fatty acid content within particular groups of phospholipids.
Our findings suggest that decreased activity of SCD1 affects autophagic flux and exacerbates palmitate-induced apoptosis in INS-1E cells by a mechanism that triggers perturbations in cellular membranes integrity and fluidity.

Acknowledgements

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O5.3

Influence of new oxicam analogues on the properties of lipid bilayers

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Piroxicam, from the group of the oxicams, is maliny known as non-steroidal anti-inflammatory drug (NSAID), used in the treatment of chronic rheumatic diseases. The molecular target of NSAIDs is cyclooxygenase (COX), the enzyme that catalyses the conversion from arachidonic acid to prostaglandins (PGs). There are three isoforms of COX (Cox-1, Cox-2 and Cox-3).
Most solid tumors express the cyclooxygenase-2 (COX-2) protein, which is a target of NSAIDs, that is why those drugs are evaluated as anti-cancer. They inhibit proliferation, invasiveness of tumors, and angiogenesis and overcome apoptosis resistance in a COX-2 dependent and independent manner [1]. Moreover, chronic inflammatory processes affect all stages of tumour development, and there are many molecular and cellular pathways that participate in the crosstalk between cancer cells and inflammatory mediators [2].
Lenard Lichtenberger *et al.* presume that one of the alternative mechanisms by which NSAIDs can be effective is by interacting with cellular membranes and altering their biophysical properties. Those drugs can induce changes in the fluidity, permeability and biophysical properties of cell membranes [3]. Also Marlene Lúcio *et al.* considers the interaction of NSAIDs with membrane models concluding that in order to achieve their main target – membrane associated enzyme COX – NSAIDs have first to pass through the membranes, that is why understanding this interaction plays a key role in understanding the therapeutic effects of those drugs [4].
To optimize and modulate the biological effects of piroxicam, some efforts are made to synthesize the derivatives of this oxicam. In present work we describe the results of calorimetric and fluorescence spectroscopic experiments of three newly synthesized analogues of piroxicam, named PR1, PR2 and PR12 on the phase behaviour of phospholipid bilayers and fluorescence quenching of two fluorescent probes – Laurdan and Prodan – which molecular location within membranes is known with certainty.
The results presented allow the conclusion that studied oxicam analogues interact with the lipid bilayers and may penetrate the membranes.

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O5.4

Susceptibility versus resistance of insects to fungal infection may result from differential lipolytic rates of their cuticles by fungal lipases affected by some epicuticular lipids

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Entomopathogenic fungi are important natural regulatory factors of insect populations and have potential as biological control agents of insect pests. The cosmopolitan soil fungus *Conidiobolus coronatus* attacks susceptible insect species (25 from 43 tested so far) due to combination of mechanical pressure on insect cuticle and production of proteases, chitinases and lipases degrading main components of insect cuticle. Prompt death of invaded insects is attributed to the action of toxic metabolites released by the invader impairing victims' immune system. Susceptibility or resistance of various insect species to fungal invasion may result from several factors, including structure of exoskeleton and composition of the epicuticular lipids as some of them significantly decrease lipolytic activity of *C. coronatus* when added to the culture medium.

The aim of this study was to check correlations between the lipolysis rates of cuticles dissected from 5 insect species with known susceptibility/resistance responses to *C. coronatus* and composition of epicuticular lipids determined by means of HPLC-LLSD and GC-MS techniques. Hydrolysis of homogenized cuticles collected from larvae, pupae and adults of *Calliphora vicina*, *Calliphora vomitoria*, *Lucillia sericata*, *Musca domestica* and *Galleria mellonella* was performed with the use of *C. coronatus* post-incubation medium showing high lipolytic activity. After 8 h incubation of cuticle preparations with enzymes released by fungus into culture medium, quantities of free fatty acids were determined fluorometrically.

Obtained data show species- and developmental stage-specific differences in the lipolysis rates. Assuming that $C_{17:1}$ fatty acid,strongly inhibiting *C. coronatus* lipases *in vitro*, was found only in *L. sericata* larval and pupal epicuticle, lack of lipolysis of *L. sericata* larval cuticle, very low rates in the case of pupal cuticle contrasting with high rates in case of cuticle from adults, suggest crucial role of this fatty acid in resistance against *C. coronatus*. On the other hand observation that cuticular lipids from 3 other resistant fly species are digested *in vitro* by fungal lipases with similar efficiency as lipids from the cuticle of highly sensitive *G. melonella* larvae, indicates complexity of mechanisms underlying insect resistance to fungal infection.

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O5.5

Lysophosphatidic acid as a regulator of lipopolysaccharide-induced signaling pathways in macrophages

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Lipopolysaccharide (LPS) belongs to glycolipids which built the outer membranes of Gram-negative bacteria. An appearance of LPS in tissues upon bacterial infection activates pro-inflammatory responses of immune cells aiming to eradicate the bacteria. Detection of LPS and triggering of the pro-inflammatory signaling in macrophages requires coordinated interactions of many proteins which bind and eventually translocate LPS onto the TLR4/MD-2 receptor complex to induce downstream signaling cascades. Activated TLR4 receptor triggers two signaling pathways which engage either MyD88 or TRIF adaptor proteins. The MyD88-dependent cascade leads to generation of pro-inflammatory cytokines, like TNF-α, while the TRIF-dependent cascade induces production of type I interferons and some chemokines, like RANTES. Since exaggerated responses to LPS result in sepsis, regulation of LPS-induced signaling is an important issue. It was suggested that lysophosphatidic acid (LPA) alters activity of immune cells and affects LPS-induced signaling cascades leading to an enhancement or a down-regulation of pro-inflammatory responses of various cells. There are nine plasma membrane receptors of LPS identified thus far which can account for the disparate results of LPA activity in those cells. We aimed to reveal the role of LPA as a modulator of LPS-induced pro-inflammatory responses of macrophages by examining production of cytokines in J774 and RAW265 macrophage-like cells. It was found that the effect of LPA on LPS-induced signaling was well pronounced in J774 but not in RAW264 cells which seems to be connected with different profiles of expression of LPA receptors in these cells. In J774 cells, the two LPS-induced signaling pathways were not equally sensitive to LPA regulation. The MyD88-dependent signaling cascade was down-regulated by LPA, judging from a reduced production of TNF-α in cells pretreated with LPS prior to stimulation with 100 ng/ml LPS. TRIF-dependent pathway was affected by LPA to lower extend since LPS-induced production of RANTES chemokine was moderately inhibited in the presence of the lipid. Our result indicates that LPA belongs to negative regulators of LPS-induced pro-inflammatory activity of macrophages.

O5.6

Consequences of cholesterol accumulation and its effects on mitochondrial function in Niemann-Pick type C disease

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Niemann Pick type C (NPC) is one of autosomal recessive diseases called lysosomal storage disorders. It is caused by the mutation in *NPC1* gene encoding protein which participates in transport of lipids from late endosomes to lysosomes. The impairment of this transport may result in cholesterol and glycolipids accumulation in the late endosomal/lysosomal (LE/LY) compartment of NPC cells [1]. Furthermore, properties of the LE/LY compartment are largely impaired in comparison to the control cells [2-5].
In this study we analyzed the morphology and function of mitochondrial network in NPC cells. As an experimental model we used fibroblasts from healthy volunteers and fibroblast cell lines from patients suffering from Niemann-Pick disease type C. These cell lines were tested to confirm the NPC phenotype. To examine the possible effect of abnormal cholesterol accumulation on mitochondria and its potential influence on the cellular energy metabolism we used several methods including measurement of mitochondrial membrane potential, oxygen consumption and finally ATP and reactive oxygen species production.
Our results revealed that NPC cells are characterized by a decreased level of NPC1 protein and an increased level of cholesterol, which accumulated in the perinuclear region of the cell. This was accompanied by changes in the mitochondrial network and reorganization of the LE/LY compartment, where mitochondria are located among vesicles with accumulated cholesterol. Functional analysis of mitochondria in NPC cells showed significantly higher oxygen consumption, lower ATP level and decreased ROS production which might suggest problems with synthesis of ATP. Functional and structural differences between NPC and control cells may suggest a complex of molecular mechanisms of which tend to be not only limited to the perturbed lipids distribution but also involved in the cellular energy metabolism.

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Posters

P5.1

Serum oxidized low-density lipoprotein (ox-LDL) in relation to circulating adiponectin in tobacco smoking and non-smoking pregnant women

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Objective: Adiponectin may protect the endothelium against the detrimental effect of oxidized low-density lipoprotein (ox-LDL). Because cigarette smoke contains reactive oxygen species, it has been hypothesized that some adverse effects of smoking may result from oxidative damage to endothelial cells in utero-placental unit.Therefore in the present study, we examined whether serum ox-LDL levels differ among smoking and non-smoking pregnant women, and whether this differences correlate with adiponectin.
Methods: Seventy five healthy pregnant women with singleton pregnancies (39–42 week of gestation), attending the Institute of Mother and Child in Warsaw were divided into smoking and tobacco abstinent group according serum cotinine concentration. The current smokers were defined as those who had smoked 5 cigarettes per day for 2 years before conception and continued smoking during pregnancy. All pregnant volunteers signed a written informed consent form, approved by the Institute's Ethical Committee. Serum concentrations of cotinine, ox-LDL and adiponectin were determined using an enzyme immunoassay. Statistical analysis was done using the STATISTICA 10.0 and the significance level was set at $p<0.05$.
Results: Mean oxidized low-density lipoprotein concentrations in the serum were significantly higher in the smoking group compared with the non-smoking one (p0.01). In contrary, the serum adiponectin levels of the smoking mothers were significantly lower than in tobacco abstinent group (p0.05). The negatve correlation between adiponectin and ox-LDL concentrations were found (r=-0.38; p0.05). In the group of smokersthere was a significant relationship between serum adiponectin valueand the number of cigarettes smoked per day ($r=0.40$, $p<0.05$) as well as serum ox-LDL value and cotinine concentration (r=0.39, p0.05).
Conclusion: Tobacco smoking during pregnancy affects serum ox-LDL as well as adiponectin values. The association of high serum adiponectin concentrations with low circulating oxidized low-density lipoprotein levels may suggest participation of this protein in the defense against oxidative stress markers. Further studies on the factors influencing the inactivation or removal of circulating ox-LDL are necessary.

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P5.2

Circulating oxidized low-density lipoprotein in obese children

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Background: Lipid peroxidation products are formed during normal cell metabolism via producing an excess of free radicals that can react with unsaturated fatty acids, in particularly low-density lipoprotein (LDL). Oxidation of this protein is considered a key factor in the pathogenesis of atherosclerosis and cardiovascular disease. In adults, elevated circulating oxidized LDL are associated with obesity, insulin resistance, metabolic syndrome, and cardiovascular disease, but little is known about its relation to obesity in childhood. Studies in adults have also shown reduction in plasma markers of oxidative stress after weight loss induced by diet, pharmacologic therapy, and surgery.
Objective: The aim of this study was to investigate the effect of lifestyle intervention on plasma oxidized LDL (ox-LDL) in prepubertal obese children.
Methods: Thirty-two obese children (age, 7.8±1.5 years; boys, 47%) before and after the 3-months intervention program were included in the study. The recommended daily energy intake was 1200-1400 kcal/day. We assessed the average daily energy intake and the percentage of energy intake from protein, fat and carbohydrates in the diets of obese and non-obese children. Physical examination was performed and body mass index (BMI) was calculated. Healthy normal-weight children (n=20) were the reference group. Children were classified as obese (SDS-BMI >2) and non-obese (SDS-BMI). The concentration of oxLDL was determined in serum by ELISA assay. Total cholesterol, LDL- and HDL-cholesterol and triglicerydes levels were measured by enzymatic methods.
Results: The concentration of oxLDL in obese children was higher by about 50% (p0.01) than in normal-weight controls. The level of this protein decreasd in obese patients after 3-months therapy by about 20% (p0.01), but was still higher than in controls. The oxLDL/LDL-cholesterol ratio was higher in obese children before therapy and sinificantly (p0.05) decreased after intervention. We observed decrease of BMI by about 10% (p0.05) in obese children after therapy. Significant positive orrelation was foud between oxLDL level and BMI ($r= 0.36$, $p<0.05$) in obese patients. No correlations were observed between ox-LDL and total cholesterol, HDL- and LDL-cholesterol.
Conclusions: Our preliminary study demonstrates that increased oxLDL is associated with obesity during prepubertal period. These changes were partially reversible by 3-months program of lifestyle modification that included diet and exercise.

P5.3

Studies on lipogenesis in the shrimp *Crangon crangon* — application of metabolic labeling with tritiated water

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The measurement of lipogenic activity is of critical importance and numerous methods have been developed aimed at evaluation of biochemical parameters of lipogenesis.
Only few publications depict the application of chromatographic methods to evaluate the mechanism of incorporation of tritium into fatty acids, so we decided to use the gas chromatography-mass spectrometry (GC-MS) to the identification and quantitative determination of the tritiated products of *de novo* lipid synthesis.
In our work brown shrimps *Crangon crangon* were used as a model for the determination of *de novo* lipogenesis and identification of the products of this process. Total lipids from muscle and hepatopancreas of *C. crangon* were extracted using the Folch method and divided into two parts. After completion of evaporation the first part of extracted fatty acids (Fas) was dissolved in scintillation fluid. Radioactivity was measured after 1h, and every subsequent hour up to 8h of incubation of shrimps with ^3H_2O, using Beckman LS scintillation 6000 IC counter. The second part of lipid extract was hydrolyzed and derivatized to the respective FA esters, and then analyzed by GC-MS. The highest intensity of lipogenesis was detected in hepatopancreas, and value of the radioactivity was 750 ± 67 dpm/100 mg tissue. In muscle tissues intensity of fatty acids synthesis was two times lower. In the chromatograms FAs with 7, 6 and 5 tritium atoms were detected, while the molecules with lower number of tritium atoms were not detected. The products with higher number of tritium atoms dominated in analyzed samples. The content of tritiated FAs was 2.3 ± 0.1%; 2.1 ± 0.1% and 0.2 ± 0.0%, of the total fatty acids for $16{:}0_T$, $18{:}0_T$, $20{:}0_T$, respectively. The highest content of total (non-tritiated) FAs among shrimp lipids was noted for highly polyunsaturated fatty acids (PUFAs), such as 20:5n-3 (EPA), 20:4n-6 (AA) and 22:6n-3 (DHA) constituting 26.1 ± 1.6%, 3.7 ± 0.2% and 1.9 ± 0.1%, respectively of the total FA pool. However, neither tritiated PUFAs nor monounsaturated fatty acids (MUFAs) were detected in shrimp lipid samples by GC-MS. This was probably due to low level of endogenous synthesis of MUFAs and PUFAs, since in shrimps these fatty acids maybe originate mainly from exogenous sources.
The application of metabolic labeling with tritiated water followed by GC-MS enables determination of the *de novo* lipogenesis in tissues of *C. crangon* and evaluation the content of specific products of this process.

P5.4

Preeclampsia-associated alterations in glycerolphospholipid composition of the human umbilical cord artery

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Preeclampsia is the most common pregnancy-associated pathological syndrome. It is accompanied by significant remodelling of the extracellular matrix and alteration in lipid composition of the umbilical cord vessels. We evaluate some glycerolphospholipid composition of umbilical cord artery (UCA) and its alteration in preeclampsia. Studies were performed on the UCAs taken from 10 newborns delivered by healthy mothers (control material) and 10 newborns delivered by mothers with preeclampsia (preeclamptic material). Solid phase extraction, thin layer chromatography and high-performance liquid chromatography were employed for these analyses.
The control UCA wall contained several glycerolphospholipids. Preeclampsia was associated with slight but statistically significant increase in total phosphatidylserine content with simultaneous decrease in phosphatidylethanolamine content. It was found an increase in total content of phosphatidylcholines and especially lysophosphatidylcholines in preeclamptic UCAs in our earlier studies. Also a marked elevation in sphingomyelin with a slight reduction in ceramide content was observed. Taking into account the decrease in DNA content it seemed an accumulation of phoshpolipids in preeclamptic UCAs. It could make easier signal transduction into the cells and, in consequence, stimulation for observed overproduction of extracellular matrix components in preeclamptic umbilical cord arterial wall.

P5.5

Fatty acids present in insect epicuticle affect activity of entomopathogenic fungus *Conidiobolus coronatus* enzymes engaged in cuticle degradation

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Naturally occurring entomopathogenic fungi are important regulatory factors of insect populations. They invade insects through the cuticle, composed of proteins and chitin covered by lipid layer, by a combination of mechanic pressure of growing hyphae and enzymatic degradation. Insecticidal fungi produce several cuticle degrading proteases, chitinases and lipases. Although mechanisms of enzymatic degradation of cuticle are intensively studied, the reasons of insects' differential susceptibility to fungal infection remain obscure.
The little investigated soil entomopathogenic fungus *Conidiobolus coronatus* effectively kills susceptible insects by secretion of cuticle degrading enzymes: elastase, chitobiosidase, NAGase, lipase as well as numerous toxic metabolites impairing victim's immune system. From 43 insect species exposed to *C. coronatus* 25 were efficiently infected while 18 showed high resistance. Susceptibility or resistance of various insect species to fungal invasion may result from the composition of cuticular lipids as some of them affect growth, sporulation and insecticidal properties of *C. coronatus* when added to the culture medium.
In present study 16 cuticular fatty acids (CFAs) identified by HPLC-LLSD and GC-MS techniques in 7 insect species with known susceptibility/resistance responses to *C. coronatus*, were added to the fungus culture medium at the minimal and maximal physiological concentrations detected in tested insects. Activities of elastase, chitobiosidase, NAGase and lipase in postincubation media were determined spectro- and fluorometrically, respectively.
Our results show that CFAs strongly influenced activities of all tested enzymes. Elastase activity increased after addition of $C_{13:0}$, $C_{17:0}$ or $C_{14:1}$ but decreased in the presence of $C_{22:0}$, $C_{24:0}$, $C_{26:0}$ or $C_{20:2}$. Inhibiting effects of most tested CFAs on NAGase and chitobisidase activities were distinct. It should be noted that $C_{14:1}$, $C_{17:1}$ and $C_{20:2}$ considerably reduced lipolytic activity.
We showed for the first time which CFAs at physiological concentrations inhibit and which stimulate activities of enzymes secreted by *C. coronatus*. Surveillance that several CFAs present in insects resistant to *C. coronatus* showed *in vitro* inhibitory effects on fungal enzymes proves their role in protecting insects from fungal invasion.

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P5.6

Structural and functional analysis of ATP-dependent ligase VinN involved in vicenistatin biosynthesis

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Adenylation enzymes play important roles in the biosynthesis and degradation of primary and secondary metabolites. They are usually found as an adenylation domain of the nonribosomal peptide synthetases (NRPS), the acyl-CoA synthetases and the luciferase enzymes. NRPS-type enzymes are responsible for selecting and activating the required substrate with high specificity and employ ATP to adenylate the carboxyl groups of amino acids as AMP-esters. The biological activity of the natural products produced by NRPSs are determined by substrates that are bound by the A (adenylation) domains.

Vicenistatin is a macrolactam polyketide antibiotic produced by *Streptomyces halsedii* HC34 and possesses unique β-amino acid starter unit at its polyketide chain elongation. The unusual findings in vicenistatin biosynthetic pathway are protection-deprotection strategy and presence of important ATP-dependent ligases (adenylation enzymes), VinN and VinM. VinN recognizes (2S,3S)-3-methylaspartate (3-MeAsp) as a β-amino acid. This property of VinN is distinct from those of well-known α-amino acid – activating enzymes.

No crystal structure of β-amino acid activating ATP-dependent ligases has been reported so far. Therefore, the β-amino acid recognition mechanism remains elusive. To clarify how VinN selectively recognizes 3-MeAsp, we determined the crystal structure of VinN. Structural and mutational analysis of VinN revealed substrate recognition mechanism and interaction between adenylating enzyme and β-amino acid.

Reference: Shinohara Y *et al* (2011) *Journal of the American Chemical Society* **133**: 18134-18137