SESSION 13

Metabolism of lipids

Organized by J. Świerczyński, W. Bogusławski, W. Trzeciak
Biosynthesis of cholesterol in the chronic renal failure
Wojciech Bogus³awski¹, Micha³ Chmielewski²
¹ – Katedra i Zak³ad Chemii Medycznej, Akademia Medyczna w Gdañsku, ul. Dêbinki 1, 80-211 Gdañsk, ² – Katedra i Klinika Nefrologii, Transplantologii i Chorób Wewnêtrznych, Akademia Medyczna w Gdañsku, ul. Dêbinki 7, 80-211 Gdañsk

Changes in lipid metabolism are an important risk factor of the vascular complications during chronic renal failure (CRF). In experimental CRF (5/6 nephrectomy model) hypercholesterolemia has been found to be main lipid disorder. Evaluation of mechanisms leading to hypercholesterolemia associated with CRF appears to be of importance. Recently we have shown that enhanced in CRF liver cholesterologenesis is at least in part, due to the increased activity of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase). Analysis of HMG-CoA reductase mRNA shows that higher activity of this enzyme in CRF rats may result, at least in part, from the increased expression of HMG-CoA reductase gene. In contrast to puromycin induced nephrotic syndrome, the diurnal rhythm of cholesterologenesis in CRF rats is preserved both in liver as well as in the rat intestinal tissues. In addition we observed higher activity of HMG-CoA reductase both at light and a dark phases of the diurnal cycle in the CRF animals.

Patients, as well as laboratory animals with CRF exhibit higher plasma mevalonate concentrations with concomitant decrease in its urinary excretion. Higher plasma level of this direct product of HMG-CoA reductase activity parallel high concentrations of plasma and liver cholesterol. Therefore increased expression of HMG-CoA reductase activity as well as HMG-CoA reductase gene and in consequence increased cholesterologenesis in the course of CRF are a surprising observations, since in the presence of elevated serum mevalonate as well as cholesterol, they would be inhibited rather than stimulated.

Moreover, starvation is one of the physiological metabolic states which cause depression of liver cholesterologenesis. Despite that, even significant decrease in food intake and body weight found in CRF animals was accompanied by higher activity of HMG-CoA reductase together with higher concentration of plasma and liver cholesterol as well as hepatic and intestinal cholesterologenesis. Therefore it seemed obvious that, as was suggested by Pandak, normal feedback regulation of HMG-CoA reductase was altered in CRF rats.

In contrast, experiments in which both control and CRF rats were kept on the normal and cholesterol supplemented diet indicate that the feedback inhibition by dietary cholesterol is present in control as well as in CRF animals.

It is important to note that the marked increase of sterol biosynthesis from mevalonate (as well as acetate) has been found in CRF rats livers. These results indicate that the increased cholesterologenesis found in the course of CRF is in fact more complex in nature than simply due to the increased activity of HMG-CoA reductase.

Adiponectin — the molecular link between obesity and diabetes
Zdzis³aw Kochan
Katedra i Zak³ad Biochemii, Akademia Medyczna w Gdañsku, ul. Dêbinki 1, 80-211 Gdañsk

Insulin resistance — a major risk factor for the development of type 2 diabetes, hypertension, and coronary artery disease is frequently associated with obesity. However, the molecular link between increased adiposity and reduced sensitivity of target tissues to insulin is far from being clear. In recent years, it has been demonstrated that proteins secreted by adipocytes, such as leptin, angiotensin 2, plasminogen-activator inhibitor-1, resistin and tumour necrosis factor-alpha, may influence insulin sensitivity. Recently, it has been reported that another fat-derived protein, adiponectin, may act as an insulin-sensitizing factor, replenishment of which increases insulin sensitivity in different models of insulin resistance.

Adiponectin, also known as an adipocyte complement-related protein of 30 kDa (Acrp30), is a novel adipocyte-specific secretory protein produced exclusively in adipocytes and secreted into the blood stream. Adiponectin gene expression is induced during adipocyte differentiation and its secretion is stimulated by insulin. It has been observed that plasma adiponectin levels are decreased in obese humans, despite increased adiposity. Furthermore, low plasma adiponectin levels are associated with insulin resistance and
hyperinsulinaemia. A genome scan provided strong evidence for a susceptibility locus for type 2 diabetes and related metabolic syndromes on chromosome 3q27, where the adiponectin gene (apM1) is located. Administration of adiponectin to mice improves glucose tolerance and insulin sensitivity. Conversely, heterozygous and homozygous adiponectin knock-out mice develop insulin resistance. Injection of adiponectin decreases plasma glucose levels by suppressing glucose production in the liver and reduces fatty acid levels by stimulating fatty acid oxidation in muscle.

With recent advances in the understanding of its function, adiponectin can now be added to the list of adipocyte-derived hormones that play important role in the regulation of glucose and lipid metabolism. The ability of adiponectin to act as an insulin sensitizing factor has made this novel protein a promising therapeutic tool for the future.

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**Regulation of hormone-sensitive lipase (HSL) in skeletal muscle cells**

Józef Langfort, M. Donsmark, T. Ploug, C. Holm, H. Galbo

1 – Laboratory of Experimental Pharmacology, Polish Academy of Science, Warszawa, 2 – Copenhagen Muscle Research Centre, Rigshospitalet, Copenhagen, Denmark, 3 – Department of Medical Physiology The Panum Institute, University of Copenhagen, Copenhagen, 4 – Department of Cell and Molecular Biology, Lund University, Lund, Sweden

Triacylglycerol (TG) is stored in lipid droplets in the cytoplasm of skeletal muscle. They can be mobilized by catecholamines, exercise and electrical stimulation. The exercise-induced decrease of TG can be reduced by beta-adrenergic blockade. The effect of catecholamines on intramuscular TG is compatible with a role of hormone sensitive lipase (HSL) in muscle. The enzymatic regulation of intracellular TG hydrolysis in skeletal muscle until recently has not been elucidated.

Then the presence of HSL, which controls TG breakdown in adipose tissue, was demonstrated in all muscle types by Western blotting muscle fibers isolated by collagenase treatment or after freeze-drying. The content of HSL varies between fiber types, being higher in oxidative than glycolytic fibers. Analysed under conditions optimal for HSL, neutral lipase activity in muscle is stimulated by adrenaline and contractions. These increased are abolished by presence of anti-HSL antibody during analysis. Immunoprecipitation with affinity-purified anti-HSL antibody causes similar reduction in muscle HSL protein concentration and in measured neutral lipase responses to contractions. Adrenaline stimulates HSL via beta-adrenergic activation of protein kinase A (PKA). Contraction stimulates HSL activity by protein kinase C (PKC) via extracellular signal regulated kinase (ERK). Adrenaline and contraction enhance muscle-HSL activity by phosphorylation. Phosphorylation of different sites explains that in muscle effect of contraction and adrenaline on HSL activity is partially additive. In line with the view that the two stimuli act by different mechanism, training increases the contraction-mediated, but diminishes the adrenaline mediated HSL activation in muscle.

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**Chronic renal failure-associated hypertriglyceridemia: Its reasons and the consequences**

Bolesław Rutkowski, Marek Szołkiewicz

Katedra i Klinika Nefrologii, Transplantologii i Chorób Wewnętrznych, Akademia Medyczna w Gdańsku, ul. Dębinki 7, 80-211 Gdańsk

High prevalence of hypertriglyceridemia (HTG) in chronic renal failure (CRF) was reported for the first time more than 30 years ago and since then it was claimed a metabolic consequence of the disease. HTG is important at least of two reasons: it is partially responsible for the accelerated atherosclerosis found in patients with CRF and moreover, it is presumed to stimulate the progression of CRF. Serum triglyceride (TG) concentration is a function of its synthesis and removal. There is strong evidence, which let us state that TG catabolism in renal failure is impaired. The activity of two enzymes highly involved in this process — lipoprotein lipase (LPL) and hepatic lipase (HL) — is reduced in renal failure and it is believed that this is due to a high concentration of lipase inhibitors in uremic plasma. Also, the VLDL receptor gene expression in different tissues is found to be down-regulated. On the other hand, there are also reasons, which let us suppose that HTG found in renal fail-
ur is not solely a result of impaired TG removal and the increased TG biosynthesis might contribute to this derangement. It appeared that in most reports it was impossible to indicate a significant correlation between serum TG concentration and the activity of lipoprotein and/or hepatic lipase (or postheparin plasma lipolytic activity). Also, the LPL activity is already decreased in early stages of CRF and HTG is rather rare at this point. Moreover, experimental studies indicate that fatty acid synthase (and some other lipogenic enzymes) gene expression is up-regulated in CRF.

Concluding, all these data confirm the thesis that renal failure — associated HTG is the result of both impaired TG catabolism and increased TG production.

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Lecture

Ftz-F1 gene expression is regulated at the transcriptional level by signal transduction pathway involving adenylyl cyclase and cyclic-adenosine monophosphate

Wies³aw Trzeciak¹, T. Lehmann¹, Justyna Biernacka-Łukanty¹, N. Saraco², D. Langlois², J. Saez², J. Li²

¹ — Katedra i Zak³ad Biochemii i Biologii Molekularnej, Akademia Medyczna im. K. Marcinkowskiego, ul. Œwiêcickiego 6, 60-781 Poznañ, 2 — Faculte de Med Laennec, INSERM U-369, Lyon, France

The Ftz-F1 gene encodes an orphan nuclear receptor SF-1 (Ad4BP, Nr5a1) that regulates transcription of a number of genes involved in the development of the adrenal cortex and gonads, as well as steroidalogenic genes controlled by protein kinase A (PKA) pathway. To date, however, the regulation of Ftz-F1 gene expression has not been thoroughly investigated.

The aim of this study was to evaluate the effect of stimulation of protein kinase A pathway by an ACTH or an activator of adenylyl cyclase, forskolin on the level of Ftz-F1 gene transcript and protein product in a subclone of Y-1 cells highly responsive to ACTH.

Treatment of these cells with ACTH or forskolin resulted in an increased CYP11A1 and Star expression. Northern blot analysis revealed 3.5-fold higher expression of Ftz-F1 after 6 h of incubation with forskolin. This was accompanied by a significant elevation of SF-1 protein level, measured by Western blotting. The accumulation of SF-1 was followed by an increase in CYP11A1 gene transcript with a maximal expression after 24 hours of treatment. The stimulatory effect of forskolin on Ftz-F1 expression and the level of protein product of the gene was abolished by actinomycin-D, suggesting that the effect of forskolin was due to stimulation of gene transcription and not to the gene transcript or protein stability.

In order to demonstrate that stimulation of Ftz-F1 gene expression can affect transcription of SF-1-controlled genes, Ftz-F1 gene was overexpressed in Y-1 cells. In these cells, co-transfected with the recombinant vector harbouring StAR promoter and luciferase gene, significant enhancement of the reporter gene expression was observed in the absence of forskolin.

It was concluded that stimulation of Ftz-F1 gene expression by PKA pathway involving ACTH receptor, adenylyl cyclase and cAMP could provide a novel important regulatory step in steroidalogenic gene expression in Y-1 cells.

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Oral Presentation

Role of calcium-independent phospholipase A2 in the UV-induced apoptosis in Jurkat cells

Wojciech Brutkowski, Krzysztof Zab³ocki, Anna Bielak, Katarzyna Piwocka, Lech Wojtczak, Jerzy Duszyñski

Zak³ad Biochemii Komórki, Instytut Biologii Doœwiadczalnej PAN im. M. Nenckiego, ul. Pasteura 3, 02-093 Warszawa

Phospholipases A2 (PLA2s) catalyze hydrolysis of the ester bond in position 2 of glycerol moiety in various types of phospholipids. These enzymes are involved in many cellular processes such as inflammation, signal transduction and membrane phospholipid turnover. PLA2s are divided to three main subclasses: sPLA2, cPLA2 and iPLA2. They differ in their molecular mass, Ca²⁺ requirement, substrate specificity and function. iPLA2 is a calcium-independent isoform and is believed to be responsible for the remodeling of cellular membranes. Moreover, there are some data indicating that this enzyme may be required for mitochondrial steps of apoptosis to occur in postischemic cardiomyocytes and also in the development of fully expressed staurosporin-induced apoptosis of lymphocytes.
The aim of this study was to test the concept that iPLA2 plays a role in the development of apoptosis in Jurkat lymphoidal cells exposed to UV light. Jurkat cells illuminated with UV exhibit typical symptoms of apoptotic death such as chromatin condensation, DNA fragmentation, activation of caspase 3 and externalization of phosphatidylcholine. Preincubation of these cells with Helss (a specific inhibitor of iPLA2) does not protect them against the loss of plasmamembrane asymmetry (visualized by annexin V binding to the cell surface) as well as from an increase in caspase 3 activity and chromatin condensation. However, the cells preincubated with Helss and then exposed to UV do not exhibit DNA degradation. This was evidenced using following approaches: (a) cell staining with Hoechst fluorescent dye, (b) flow cytometry measurements of the “sub-G1” fraction, and (c) flow cytometry TUNEL test.

We conclude, that iPLA2 activity is involved in the activation of DNA fragmentation, but it is not necessary for other steps of apoptosis to progress in Jurkat cells exposed to the UV.

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**Influence of sugar and sodium fluoride on fatty acid metabolism in isolated organs of yellow lupine cultivated in vitro**

Sławomir Borek, Maciej Doszczeczko, Wiktoria Ratajczak, Lech Ratajczak

Department of Plant Physiology, A. Mickiewicz University, ul. Niepodległości, 61-713 Poznań

Recent studies have shown that storage lipids in yellow lupine seedlings are converted not only to sugar, but also to amino acids (Borek et al. 2003, J Plant Physiol, 160(5): 539–545). Our results concern the directions of storage lipid metabolism in isolated organs of yellow lupine, and the influence of sugar and sodium fluoride on these processes.

Isolated embryo axes and isolated cotyledons, cultivated for 96 hours *in vitro* on a medium supplemented with 60 mM saccharose or without the sugar, were incubated for 120 minutes in a solution of 1-14C- or 2-14C-labelled acetic acid with sodium fluoride and without NaF. The radioactivity of liberated 14CO2 was measured, and incorporation of 14C into selected amino acids and sugars was analyzed using paper chromatography.

The release of 14CO2 in the embryo axes and cotyledons were considerably higher from 1-14C-labelled acetic acid. The 2-14C of acetic acid was incorporated more intensively into amino acids (mainly aspartate, asparagine, glutamate, and glutamine) and sugars (mainly glucose). Higher amounts of 14CO2 were released when the organs were cultivated on the medium without saccharose. Both, 1-14C and 2-14C were not incorporated into the sugars of axes grown on the medium without saccharose. Incorporation of the 2-14C of acetic acid into amino acids of the axes and cotyledons was higher when the organs were cultivated on the medium with saccharose. On the other hand, the sugar did not affect incorporation of the 1-14C of acetic acid into amino acids. Sodium fluoride added to the incubation mixture slightly lowered the amount of 14CO2 released from the axes and cotyledons regardless of the trophic conditions of the culture, however, it considerably limited incorporation of both 1-14C and 2-14C into the aforementioned amino acids and all sugars. The saccharose in the culture medium did not influence incorporation of the 1-14C of acetic acid into amino acids when sodium fluoride was added, however, more radioactivity from the 2-14C was localized in the amino acids of axes and cotyledons grown on the medium without saccharose.

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**The growth of rsp5 mutants of *S. cerevisiae* is affected by multicopy PIS1 gene encoding phosphatidylinositol synthase**

Paweł Kaliszewski, Beata Gajewska, Joanna Kamińska, Teresa Żołędak

Instytut Biochemii i Biofizyki, Polska Akademia Nauk, ul. Puławskiego 5A, 02-106 Warszawa

Rsp5 ubiquitin ligase has essential role in biosynthesis of unsaturated fatty acids and unessential roles in endocytosis and other processes in yeast. To gain better understanding of Rsp5p function we performed a multicopy suppressor screen and we found PIS1 gene encoding phosphatidylinositol synthase as a suppressor of rsp5 growth defect. Suppression was allele non-specific since all rsp5 mutants tested, includ-
ing rsp5Δ, were suppressed. This indicates that the mechanism of suppression probably does not involve Rsp5p and Pis1p direct interaction. Transcription of PIS1 gene was increased about 2-fold in rsp5 mutant as compared to wild type when tested by transcriptional reporter fusion on the plasmid YEP-PPIS1-lacZ or gal4::PPIS1-cat integrated to the genome. However, tested by Western blot expression of tagged HA-PIS1 from single copy plasmid was not affected in the same rsp5 mutant indicating for additional translational regulation of PIS1 expression. Pis1p was localized in the cytoplasm in foci which may correspond to cortical ER and this localization was not affected by rsp5 mutation. According to our current hypothesis PIS1 may suppress the growth defect of rsp5 by increasing the level of phosphatidylinositol which might be negatively influenced by the deficiency of unsaturated fatty acids in rsp5 cells.

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Heterogenity of precipitation subfractions of HDL

Barbara Kortas-Stempak1, Małgorzata Wróblewska2, Tadeusz Badzio3, Mirosława Szczepańska-Konkel1

1 — Katedra i Zakład Analityki Klinicznej, Akademia Medyczna w Gdańsku, ul. Dębinki 7, 80-041 Gdańsk, 2 — Katedra Biochemii Klinicznej, Zakład Medycyny Laboratoryjnej, Akademia Medyczna w Gdańsku, ul. Dębinki 7, 80-211 Gdańsk

Five HDL subfractions, named P1 to P5, were isolated according to their susceptibility to precipitation by different concentration of MnCl₂, dextrane sulphate, NaCl and pH. We found that these five subfractions differ in their phospholipid / apolipoprotein A-II (Fl/apo A-II) ratio. In P1 subfraction the Fl/apo A-II ratio was equal to 9, whereas in P5 the ratio was only 0.4. We analysed the lipid and apolipoprotein composition of P1 and P5 subfractions.

According to the hydrated density the P1 subfraction belongs to HDL2 isolated by ultracentrifugation. On the basis of apolipoprotein composition it represents the subpopulation of HDL particles containing apo-AI without apo-AII. The total concentration of P1 subfraction in serum is variable. We found that P1 subfraction is heterogeneous and consist of two different types of lipoprotein particles. One of them contains particles rich in triglycerides, with low level of cholesterol esters. The other contains particles with high amount of cholesterol esters. The proportion of these two P1 subclasses depends on total P1 concentration. When P1 concentration is low, subfraction with low level of cholesterol esters dominates. Reversibly, at high P1 concentration the participation of particles with high level of esterified cholesterol increases. We suppose, that P1 subfraction covers HDL particles acting with cholesterol ester transfer protein (CETP).

The cholesterol in P5 subfraction is esterified up to 86% — maximum possible esterification of serum cholesterol. According to apolipoprotein composition P5 subfraction is composed of three subclasses: the first contains HDL with apo A-I without A-II, the second contains HDL with both apo A-I and apo A-II, and the last contains only apo AII without apo A-I. Probably these subpopulations of particles act with HDL receptors.

We conclude, that HDL subfractions obtained by our precipitation methods represent the different stages of HDL interconversion occurring in plasma. The further investigation of these subfractions may be helpful in explanation of antiatherogenic effect of HDL.

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Feedback regulation of cholesterol biosynthesis in experimental chronic renal failure

Ewa Kossowska1, Michał Chmielewski2, Urszula Makosz1, Julian Świarczyński1, Bolesław Rutkowski2, Wojciech Bogusławski3

1 — Katedra i Zakład Biochemii, Akademia Medyczna w Gdańsku, ul. Dębinki 1, 80-211 Gdańsk, 2 — Katedra i Klinika Nefrologii, Transplantologii i Chorób Wewnętrznych, Akademia Medyczna w Gdańsku, ul. Dębinki 7, 80-211 Gdańsk, 3 — Katedra i Zakład Chemii Medycznej, Akademia Medyczna w Gdańsku, ul. Dębinki 1, 80-211 Gdańsk

Changes in lipid metabolism are an important risk factor for vascular complications during chronic renal failure (CRF). In experimental CRF hypercholesterolemia has been found to be the main lipid disorder. It is probably due to enhanced liver as well as intestinal cholesterologenesis. Our recently published results showed increased liver gene expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase) — the rate limiting enzyme in cholesterologenesis pathway, in the course of CRF. It is
known that HMG-CoA reductase activity subjects feedback regulation by exogenous cholesterol and by mevalonate. Several-fold increase of serum cholesterol and mevalonate levels has been noted in CRF animals. Increased cholesterologenesis in the course of CRF is therefore a surprising observation since in the presence of elevated mevalonate and cholesterol level, activity of HMG-CoA reductase and its mRNA level would be expected to be suppressed rather than stimulated. Therefore it was suggested by several authors that the normal feedback regulation of HMG-CoA reductase and cholesterologenesis was altered in CRF rats. The aim of the present study was to evaluate, whether cholesterol rich diet inhibits cholesterologenesis both in the liver and intestine of CRF rats. Wistar rats were used and experimental CRF was achieved by 5/6 nephrectomy model. The control as well as uremic animals were kept for three last days on the cholesterol free or 1% cholesterol supplemented diet. Activity of cholesterologenesis was investigated using tritiated water injected peritoneally one hour before sacrification. The results obtained indicate that both in the control and CRF animals, dietary cholesterol inhibits liver as well intestinal cholesterologenesis.

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Poster

Are zinc and copper involved in triacylglycerol hydrolysis and transacylation with cholesterol in men with atherosclerosis obliterans?

Anna Pioruńska-Mikołajczak¹, Maria Pioruńska-Stolzmann¹, Maria Iskra², Wacław Majewski³

¹ — Lipid Metabolism Laboratory Department of General Chemistry, Poznań University of Medical Sciences, ul. Grunwaldzka 6, 60-780 Poznań, 2 — Department of General Chemistry, Poznań University of Medical Sciences, ul. Grunwaldzka 6, 60-780 Poznań, 3 — Klinika Chirurgii Ogólnej i Naczyni, Akademia Medyczna, ul. Długa 1/2, 61-848 Poznań

Mineral imbalance in the arterial wall and serum may be an important etiological factor in the progression of atherosclerosis (Iskra et al. 1997). It is known that zinc (Zn) and copper (Cu) can affect the lipase activity and lipid concentrations in patients with atherosclerosis (Pioruńska-Stolzmann et al. 1998). Moreover, an interaction between high levels of triglyceride and cholesterol as an important agent of the assessment of cardiovascular risk in men has been postulated (Stavenov and Kjellstrom, 1999). Since triacylglycerols are not only decomposed by the lipase (GEH), but can also be transformed by transacylation with cholesterol (GECAT) in atherosclerotic men (Pioruńska-Mikołajczak et al. 2002), therefore an assessment of the role of Zn and Cu contributing in GECAT activity seemed to be interesting. The subjects of the study were 14 male inpatients with the diagnosis of femoral atherosclerosis obliterans. Samples of the aorta wall from patients were obtained at the endarterectomy. For the GEH and GECAT activities assay in serum and in arterial wall the aqueous extracts from acetone-butanol powders of proteins were analysed (Pioruńska-Stolzmann and Pioruńska-Mikołajczak, 2001). Zn, Cu and calcium (Ca) or magnesium (Mg) concentrations were determined by atomic absorption spectrometry (Iskra et al. 1997). It was found that men with atherosclerosis had higher concentrations of triacylglycerol, total- and LDL-cholesterol in serum when compared with control. Moreover, the lowering of GECAT activity in patients was observed, whereas there were no differences in GEH between patients and control. In the arterial wall of patients the higher concentration of Zn and Ca was found, whereas Cu and Mg were not affected. Additionally, the statistically significant positive correlation (r=0.55) between Zn and GECAT in patients was found. In conclusion, since GECAT activity is influenced by Zn and simultaneously the enhancement of Zn level in patients is observed, therefore these changes should be taken into consideration in evaluating the cholesterol transacylation process during atherosclerosis.

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Poster

Sphingoid derivatives of umbilical cord artery, vein and Wharton’s jelly

Lech Romanowicz¹, Zofia Galewska¹, Edward Bańkowski¹, Stefan Jaworski²

¹ — Zakład Biochemii Lekarskiej, Akademia Medyczna w Białymstoku, ul. Mickiewicza 2, 15-089 Białystok, 2 — Klinika Ginekologii, Akademia Medyczna w Białymstoku, Białystok

Sphingolipids play many biological functions, among them an important role in the maintenance of membrane structure and as second messengers. So we decided to determine sphingoid bases and their phos-
phates content in umbilical cord and serum. Studies were performed on the umbilical cord arteries, vein and Wharton’s jelly taken from 10 newborns delivered by healthy mothers. Sphingoid derivatives were isolated by Solid-Phase Extraction and analyzed by HPLC of their o-phthalaldehyde derivatives using fluorescence detection. It was found that sphingosine 1-phosphate, sphinganine 1-phosphate, sphingosine, sphinganine, 4-hydroxysphinganine galactosylsphingosine and glucosylsphingosine was present in umbilical cord and serum. The highest amount of sphingosine 1-phosphate, sphinganine 1-phosphate, sphingosine, sphinganine, 4-hydroxysphinganine galactosylsphingosine and glucosylsphingosine was observed in umbilical cord arteries. Wharton’s jelly was that umbilical cord part which contained the lowest amount of all determined sphingoid derivatives, because there was low amount of cells. Interestingly umbilical serum contained proportional high amount of sphingosine 1-phosphate which could be produced by platelets.

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**Influence of short and long term food restriction on malic enzyme gene expression in rat white adipose tissue**

Ewa Stelmańska, Justyna Korczyńska, Julian Świerczyński

*Katedra i Zakład Biochemii, Akademia Medyczna w Gdańsku, ul. Dębinki 1, 80-211 Gdańsk*

Extramitochondrial malic enzyme (ME) belongs to the family of lipogenic enzymes. The major role of this protein in liver and white adipose tissue (WAT) is the production of NADPH required for fatty acid synthesis.

The rate of fatty acid synthesis and the activities of the lipogenic enzymes are reduced with starvation. Refeeding markedly increases the activities of lipogenic enzymes and restore the capability of the tissues to form triglycerides. However little is known about the effect of food restriction on malic enzyme gene expression.

The aim of the present study was to investigate the effect of short and long term food restriction on ME gene expression in rat WAT. Male Wistar rats obtained every morning for 30 days (long term) and 3 days (short term) 85%, 70% and 50% of total amount of food consumed by control group respectively. After that all animals were allowed free access to food for 48 h.

The level of ME mRNA was much higher in all group of rats given restricted diets (as compared to control) for short and long term food restriction. The ME activity was the highest in rats receiving 70% and 50% of diet. In rats given restricted diets for 3 and 30 days the ME activity increased about three and ten times respectively. The pattern of changes in ME protein level resembles that of changes in the ME activity. This diet did not affect significantly serum glucose concentration, while insulin concentration increased in all group of rats as compared to control.

Our results show that food restriction induced malic enzyme gene expression in rat WAT. The changes in the level of malic enzyme gene expression were associated with the degrees of food restriction and the length of experiments. Increased serum insulin concentration could be responsible for increase of ME gene expresion.

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**Sex hormones effect on 6-phosphogluconate dehydrogenase gene expression in rat white adipose tissue and liver**

Tomasz Śledziński, Areta Hebanowska, Julian Świerczyński

*Katedra i Zakład Biochemii, Akademia Medyczna w Gdańsku, ul. Dębinki 1, 80-211 Gdańsk*

6-Phosphogluconate dehydrogenase (6PGDH) is one of the enzymes of the pentose phosphate shunt, a metabolic pathway producing NADPH and pentoses from glucose-6-phosphate. In liver 6PGDH activity and mRNA level in females grow with age until 9th month of life, while in males it stay unchanged during lifetime. In white adipose tissue (WAT) 6PGDH activity and mRNA level in males and females reach maximum at 2nd month of life, and than fall down. 6PGDH activity in 2 months old females is higher than in 2 months old males in WAT. These data suggest different regulation of 6PGDH gene expression in liver and WAT. It has been shown, that in liver 6PGDH gene expression is regulated by oestradiol.

The aim of the study was to reveal the influence of sex hormones on 6PGDH gene expression in WAT. For
comparison the changes of liver 6PGDH gene expres-
sion was also studied.

WAT and liver was obtained from 9 months old male
and female Wistar rats. A group of the female rats was
bilaterally ovariectomized. Some of ovariectomized fe-
male rats have received subcutaneously 17β-oestradiol.
A group of male rats was castrated. Some of castrated
male rats have received subcutaneously testosterone.
6PGDH activity and mRNA level were measured in
WAT and liver. Serum concentration of sex hormones
was measured.

In WAT of rats no correlations were found between
serum concentration of sex hormones and 6PGDH ac-
tivity and mRNA level during lifetime, whereas
changes of female liver 6PGDH gene expression corre-
lated with changes in serum oestradiol concentration.
In WAT of 9 months old rats ovariectomy or castration
did not cause significant changes in 6PGDH gene ex-
pression, whereas in liver of ovariectomized rats de-
crease of 6PGDH activity and mRNA level was found.
Administration of oestradiol and testosterone did not
cause significant changes in 6PGDH gene expression in
WAT, whereas oestradiol administration to ovariecto-
mized rats caused increase of 6PGDH activity and
mRNA levels in liver.

These data suggest that 6PGDH gene expression in
WAT is not regulated by sex hormones, whereas in
liver it is regulated by oestradiol.

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**454**

**Poster**

**Taq1B polymorphism of the cholesteryl ester transfer protein gene and CETP activity in members of families with a history of ischaemic heart disease**

Teresa Wesołowska¹, Grażyna Adler¹, Kornel Chelstowski¹, Andrzej Ciechanowicz², Barbara Torbus-Lisiecka¹, Marek Naruszewicz¹

¹ – Chair and Department of Clinical Biochemistry and Laboratory Diagnostics, Pomeranian Medical University, ul. Powstańców Wilk. 72, 70-111 Szczecin, ² – Department of Pathobiocchemistry, Pomeranian Medical University, ul. Powstańców Wilk. 72, 70-111 Szczecin

Taq1B polymorphism of the cholesteryl ester transfer protein (CETP) gene appears to influence CETP activity, as well as plasma levels of high density lipoproteins (HDL-C) and apolipoprotein A1 which are recognized markers of premature atherosclerosis. We have studied for the first time in Poland the distribution of CETP gene alleles in relation to the lipid profile and apolipoprotein levels in young 38 parents and their 60 offspring from families with a history of ischaemic heart disease (IHD). No significant effect of Taq1B CETP gene polymorphism on CETP activity in families with a history of IHD residing in Szczecin was observed. Taq1B genotype seems unrelated to the risk of coronary heart disease in children from families with IHD history. However, the B2 allele may exert an anti-atherogenic effect in young males. Tobacco smoking and B1 allele predispose to lower HDL-C levels and higher CETP activities associated with an atherogenic phenotype of HDL and LDL fractions in males. Multivariate analysis revealed that CETP activity in sons was determined by paternal CETP activity, triglyceride levels, LDL-C/HDL-C ratio, BMI, body fat percentage and water content (R=0.631; p<0.00015). CETP activity in daughters was determined by maternal total cholesterol, apolipoprotein B and triglyceride levels (R=0.570; p<0.004). Correlation analysis of CETP activity, lipid levels and body weight in offspring and their parents suggests a genetic background of the metabolic disorders and predisposition to lipid abnormalities in offspring with a family history of cardiovascular disease.

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**Poster**

**Apolipoproteins A-I and A-II dissociate from HDL and form pre-β particles when exogenous phospholipids are present**

Małgorzata Wróblewska¹, Barbara Kortas-Stempak²

¹ – Katedra Biochemii Klinicznej, Zakład Medycyny Laboratorium Klinicznego, Akademia Medyczna w Gdańsku, ul. Dębinki 7, 80-211 Gdańsk, ² – Katedra i Zakład Analizy Klinicznej, Akademia Medyczna w Gdańsku, ul. Dębinki 7, 80-041 Gdańsk

HDL consist polymorphic class of lipoproteins. Two
main subclasses are now recognised; those that contain
only apo A-I and those that contain both apo A-I and
apo A-II. While the role of apo A-I in reverse cholesterol
transport is strongly confirmed, the physiological significance of apo A-II remains unclear. Apolipoprotein A-II is synthesised in the liver as a free protein, but it occurs in plasma exclusively as a component of lipoprotein particles. The process of forming HDL apo A-I/apo A-II still is unknown.

We incubated isolated HDL with lecithin liposomes for one hour in 37°C. Agarose electrophoresis followed by immunoblotting was used to determine electrophoretic mobility of the apo A-I and apo A-II containing fractions. We observed the presence of apo A-I and apo A-II in the pre-β mobility region. We proved, that pre-β mobility fraction is not the subpopulation of liposomes, but it focuses a significant part of liposomes phospholipids. We isolated the products of the reaction from agarose gel. Polyacrylamide gel electrophoresis was used to determine molecular weight of apolipoprotein containing fractions. The pre-β fraction was composed of at least three apo A-I subfractions. Their molecular weight were assigned for about 100, 200 and 500 kDa. We observed only one 500 kDa apo-AII fraction.

We analysed the lipid and protein composition of isolated fractions. After the reaction with liposomes HDL particles lost 80% of free cholesterol, 10% of apo A-I and 5% of apo A-II.

Apo A-II is a strongly hydrophobic protein and it’s dissociation from HDL was not observed earlier. However we believe, that interaction between HDL and exogenous phospholipids causes dissociation of both apo-A I and apo A-II from HDL particles. Apolipoproteins form complexes with phospholipids and HDL free cholesterol. Our observations confirm that precursor apo A-I/apo A-II particles can be created during metabolic conversion of HDL.

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Reduction of the ob gene expression in rat white adipose tissue by triiodothyronine

Lidia Zabrocka, Jerzy Klimek
Katedra i Zakład Biochemii Farmaceutycznej, Akademia Medyczna w Gdańsku, ul. Dębinki 1, 80-211 Gdańsk

Introduction: Leptin the product of the ob gene is an important circulating signal for the control of body weight. The second an important regulator of both basal and total energy consumption is thyroid hormone – triiodothyronine (T3). One of the effect of T3 is increase in metabolic rate and increase of thermogenesis.

The aim of this study was evaluation of influence of thyroid hormones on the leptin gene expression.

Methods: Hyperthyroidism was induced by single subcutaneous injection of different doses of T3 (2 µg, 10 µg, 50 µg and 250 µg T3/100 g body weight).

Results: Obtained results indicate that T3 reduced the ob mRNA level in rat adipose tissue and serum leptin concentration. Simultaneously T3 increased lipogenic enzymes (i.e. malic enzyme, fatty acid synthase, ATP-citrate lyase, acetyl-CoA carboxylase) mRNA level.

Conclusion: These data indicate that thyroid state modulates ob gene expression and serum leptin concentration.