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VASCULAR SURGERY
PISMO POLSKIEGO TOWARZYSTWA
CHIRURGII NACZYNIOWEJ

## Carotenoids and Dietary Lipids in Health and Disease

International Conference finalizing
the DLARFID project (QLRT-2001-00183)

Kraków, 9–12 December 2004

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# Carotenoids and Dietary Lipids in Health and Disease

## International Conference
## finalizing the DLARFID project
## (QLRT-2001-00183)

Kraków, 9–12 December 2004

VENUE: Collegium Novum
The Jagiellonian University
Kraków, Gołębia 24 Str.

**The DLARFID Conference is held under the patronage of:**

Professor Franciszek Ziejka
The Rector Magnificus of The Jagiellonian University

Professor Maria Nowakowska
The Vice-Rector of The Jagiellonian University for Research and International Relations

Professor Marek Zembala
The Vice-Rector of The Jagiellonian University for the Medical College

**With invitation of the Presidents of the Societies:**

European Atherosclerosis Society (EAS) — prof. Michał Tendera
Polish Angiological Society — prof. Wojciech Witkiewicz
Polish Atherosclerosis Society — prof. Marek Naruszewicz
Polish Biochemical Society — prof. Jolanta Barańska
Polish Cardiac Society — prof. Andrzej Januszewicz
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Polish Society of Nutritional Sciences — prof. Anna-Gronowska-Senger
Polish Scientific Society for Obesity and Metabolism — prof. Barbara Zahorska-Markiewicz
Polish Union of Oncology — dr. Janusz Meder
Polish Society for Pathogenesis and Therapy of Obesity — prof. Andrzej Milewicz
Postgraduate School of Molecular Medicine — prof. Liliana Konarska

**Sponsorship:**

**FP5 DLARFID (QLK1-CT-2001-00183)** "Dietary Lipids as Risk Factors in Development. Mechanistic Issues"
**FP5 STEC (QLK3-CT-2002-30307)** "Stem Cell Therapeutics Excellence Center"
**COST-B17** "Insulin resistance, obesity and diabetes mellitus in the elderly"
**FP6-2002-FOOD 506360 NuGO** "European Nutrigenomics Organisation — Linking genomics, nutrition and health research"
**156/E-390/SP/MSN/P-05/DWM 565** "The New Technology in Medicine Center — Stem Cells Network of Excellence"
**DSM** Nutritional Products (Basel) Switzerland
Roche Diagnostics Polska sp. z.o.o
Novartis Poland sp. z.o.o
Perlan Technologies sp. z o.o.

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# Carotenoids and Dietary Lipids in Health and disease
# International DLARFID Conference

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## *Dear Colleagues*

*The nutrients, including fatty acids and lipid-soluble vitamins including Vit A, or its precursors, are essential for the development and tissue organization in any period of our life. They directly and indirectly regulate different bioactive substances gene expression, which is important for energy production, but also for cellular maturation and functional remodeling of the tissue including adipose tissue, muscle, vasculature etc.*

*Unfortunately, similar cellular mechanisms are involved in deep pathology of diet-mediated social life-threatening illnesses such as: the induction of obesity (differentiation of adipocytes), glucose and lipid intolerance (diabetes and arteriosclerosis), cancer (proliferation of non-differentiated cells) and pathological angiogenesis (cancer, arteriosclerosis), all of which impair social and health care benefits.*

*The fifth EU Framework DLARFID (Dietary Lipids as Risk Factor in Development Mechanistic Issues) was aimed at linking consumer hazards and understanding the role of nutrients such as fatty acids and carotenoids in health and disease.*

*This Symposium, organized at the end of the cooperation in frame of this project, offer the unique opportunity to bring together renomed experts (including representatives of the other EU Commission projects such as NuGO, LIPGENE and COST 17B Action) and young scientists (The Postgraduate School of Molecular Medicine (SMM)) to discuss the results, as well as build an international platform of nutrigenomic research in Europe.*

*The charming atmosphere of medieval Kraków, preparing for Christmas, is waiting for you...*

*We look forward to light the candles and sing Christmas Carols with you...*

*On behalf of the DLARFID consortium*

*Aldona Dembińska-Kieć*

# General program

## Thursday, 9 December 2004

### COLLEGIUM NOVUM (SALA SENACKA)

**9.00–15.00** **THE DLARFID CONSORTIUM MEETING**
for members DLAFRID Consortium only

### COLLEGIUM NOVUM (AULA)

**18:00** **OPENING CEREMONY**

**19:00** **FIRST PLENARY LECTURE**

**Fatty acid composition of fats as an early determinant of childhood obesity**
Gerard Ailhaud (France)

20:00 Welcome cocktail (Collegium Novum)

## Friday, 10 December 2004

### COLLEGIUM NOVUM (AULA)

**SESSION I**
**LIPIDS AND CAROTENOIDS. SOURCES, METABOLISM AND MECHANISMS OF GENOMIC AND NON-GENOMIC ACTIONS**
Chairmen: Sue Southon, Saverio Cinti

**08:45** **Challenges to understanding and measuring carotenoid bioavailability**
Sue Southon (United Kingdom)

**09:25** **Fatty acids and expression of adipokines**
Christian A. Drevon (Norway)

**10:05** **Structurally different marine oils in health and disease**
Livar Frøyland (Norway)

10:45–11:00 Coffee break

**11:00** **Mechanisms of genomic and non-genomic actions of lipids and carotenoids**
Ruan Elliott (United Kingdom)

**11:40** **Towards a better understanding of carotenoid metabolism in animals**
Johannes von Lintig (Germany)

**12:20** **Morphology of ferret subcutaneous adipose tissue after six-month daily supplementation with oral beta-carotene**
Saverio Cinti, Manrico Morroni (Italy)

13:00–14:00 Lunch break (Zodiak Hall, Św. Anny 12 Str.)

**SESSION IIA**
**CAROTENOIDS AND LIPIDS IN CELL PROLIFERATION AND DIFFERENTIATION (NORMAL CELLS)**
Chairmen: Gerd Schmitz, Andreu Palou

**14:00** **Gene regulation by beta-carotene in ferrets**
Andreu Palou (Spain)

**14:30** **Gene expression profiling identifies retinoids as potent inducers of macrophage lipid efflux**
Gerd Schmitz, Thomas Langmann (Germany)

**15:00** **Vitamin A as a regulator of adipogenesis and adipocyte metabolism-derived medical complications**
Andreu Palou, María Luisa Bonet (Spain)

**15:30** **Transgenic embryonic stem cells for basic research and clinical application**
Jürgen Hescheler (Germany)

16:00 –16:15 Coffee break

Chairmen: Jan Nedergaard, Karsten Kristiansen

**16:15** **PPAR$\gamma$ in the control of brown adipocyte differentiation**
Jan Nedergaard (Sweden)

**16:45** **Regulation of adipocyte differentiation and function by polyunsaturated fatty acid**
Lise Madsen (Norway/Denmark)

**17:15** **Molecular mechanisms controlling white versus brown adipocyte differentiation**
Karsten Kristiansen (Denmark)

**17:45** **Umbilical cord progenitor cell differentiation in the presence of PPAR-gamma and RAR/RXR activators**
Aldona Dembińska-Kieć (Poland)

19:30 Gala Dinner (Pokutynski Palace, Karmelicka 29 Str.)

## Saturday, 11 December 2004

## COLLEGIUM NOVUM (AULA)

**SESSION IIB**
**CAROTENOIDS AND LIPIDS IN CELL PROLIFERATION AND DIFFERENTIATION (CANCER CELLS)**
Chairmen: Jaap Keijer, Piotr Laidler

**08:45** **Effects of $\beta$-carotene and lycopene in cells exposed to cigarette smoke condensate: modulation of redox sensitive molecular pathways involved in cell growth**
Paola Palozza (Italy)

**09:15** **$\beta$-carotene-induced changes in RAR$\beta$ isoform expression pattern do not influence lung adenoma multiplicity in the NNK-initiated A/J mouse model**
Regina Goralczyk (Switzerland)

**09:45** **The effect of $\beta$-carotene in colon and lung epithelial cells**
Jaap Keijer (The Netherlands)

**10:15** **The effect of beta-carotene and fatty acids on proliferation and apoptosis of human melanoma and prostate cancer cells**
Piotr Laidler (Poland)

10:45–11:00 Coffee break

Chairmen: Maria Stacewicz-Sapuntzakis, John S. Bertram

**11:00** **Role of lycopene and tomato products in prostate health**
Maria Stacewicz-Sapuntzakis, Phyllis E. Bowen (United States of America)

**11:30** **The *in vitro* effect of $\beta$-carotene and arachidonic acid on cytotoxicity, proliferative potential, differentiation and apoptosis on the acute myeloid leukemia cells**
Aleksander B. Skotnicki, Tomasz Sacha, Gerd Schmitz, Thomas Langmann (Poland, Germany)

**12:00** **Cancer prevention by retinoids and carotenoids: independent action on a common target**
John S. Bertram (United States of America)

## COLLEGIUM NOVUM (HALL)

**12.30–14.00** **POSTER SESSION**

**I. Cell differentiation (signal transduction)**

1. **Prostate cancer and supplementation with fatty acid and beta-carotene: cell cycle regulation**
   J. Dulińska, P. Laidler, D. Gil, W. Placha, A. Dembińska-Kieć, G. Schmitz
2. **Different effect of beta-carotene on proliferation of prostate cancer cells**
   J. Dulińska, D. Gil, J. Zagajewski, J. Hartwich, M. Bodzioch, A. Kieć-Dembińska, T. Langmann, G. Schmitz, P. Laidler
3. **The effect of arachidonic acid and beta-carotene on human melanoma cell growth**
   D. Gil, W. Placha, J. Dulińska, A. Kieć-Dembińska, G. Schmitz, P. Laidler
4. **Retinoid signaling promoted by apocarotenals and beta-carotene in BEAS-2B human bronchioepithelial cells**
   E. Kuntz, U. Hoeller, B. Greatrix, C. Lankin, N. Seifert, S. Acharya, G. Riss, P. Buchwald-Hunziker, W. Hunziker, R. Goralczyk, K. Wertz
5. **Beta-carotene beadlets do not cause changes in RARbeta, RARalpha and PCNA expression nor in histology in ferret lungs**
   A. Wyss, V. Elste, A. Kliemant, W. Cohn, U. Hoeller, F. Ringenbach, G. Riss, E. Wolz, D. Wolff, J. Bausch, R. Goralczyk
6. **Effects of oral beta-carotene supplementation on cell cycle markers in the lungs of ferrets**
   M.A. Fuster, C. Picó, P. Oliver, J. Sánchez, A. Palou
7. **Beta-carotene stability and uptake by human lung bronchial epithelial cells depending on delivery vehicle**
   A.M. Rodriguez, S. Sastre, J. Ribot, A. Palou
8. **Comparison of influence of beta-carotene on EPC and HUVEC**
   B. Kieć-Wilk, A. Polus, J. Grzybowska, M. Mikołajczyk, J. Hartwich, T. Langmann, G. Schmitz, A. Dembińska-Kieć
9. **Proangiogenic activity of beta-carotene is coupled with the activation of endothelial cell chemotaxis**
   A. Polus, J. Grzybowska, M. Mikołajczyk, B. Kieć-Wilk, J. Hartwich, T. Langmann, G. Schmitz, A. Dembińska-Kieć
10. **Influence of squalene treatment on signal transduction**
    O. Bogdanova, L. Ostapchenko

11. **Influence of PPARγ agonists on endothelial cells differentiation and on bFGF and VEGF-dependent tubulogenesis**
    J. Grzybowska, B. Kieć-Wilk, A. Polus, E. Piątkowska, K. Kristiansen, A. Dembińska-Kieć
12. **The effect of beta-carotene on differentiation, cytotoxicity, apoptosis and proliferative potential on the three human acute leukemia cell lines**
    T. Sacha, M. Zawada, J. Hartwich, Z. Lach, A. Polus, M. Szostek, E. Zdziłowska, M. Libura, M. Bodzioch, A. Dembińska-Kieć, R. Goralczyk, K. Wertz, G. Riss, C. Moehle, T. Langmann, G. Schmitz, A.B. Skotnicki
13. **The potential of beta-carotene to influence the cell fate through the induction or inhibition of apoptosis**
    M. Bodzioch, A. Dembińska-Kieć, J. Hartwich, K. Łapicka-Bodzioch, J. Grzybowska, A. Polus, I. Wybrańska, A. Banaś, J. Dulińska, D. Gil, P. Laidler, W. Placha, M. Zawada, A. Balana-Nowak, T. Sacha, B. Kieć-Wilk, A.B. Skotnicki, C. Moehle, T. Langmann, G. Schmitz
14. **Effect of catechin and epicatechin on pyruvate dehydrogenase kinase activity in primary culture of rats hepatocytes**
    M. Tyszka-Czochara, M. Knapik-Czajka, A. Goździalska, J. Jaśkiewicz
15. **Effect of polyphenols and etanol on pyruvate dehydrogenase kinase activity in primary culture of rats hepatocytes**
    M. Tyszka-Czochara, M. Knapik-Czajka, A. Goździalska, J. Jaśkiewicz

**II. Adipocytes**

1. **UCP1 is expressed in both white and brown adipose tissues of ferrets and is down-regulated after six month daily oral beta-carotene supplementation**
   J. Sánchez, M.A. Fuster, P. Oliver, C. Picó, A. Palou
2. **Modulation of resistin expression by retinoic acid and vitamin A status**
   F. Felipe, J. Ribot, M.L. Bonet, A. Palou
3. **Retinoic acid treatment induces brown fat cell features in murine white adipose tissues**
   J. Mercader, F. Felipe, J. Ribot, A. Palou, M.L. Bonet
4. **Effect of PPAR gamma agonist and high fat diet on the content and composition of ceramides and sphingomielines in the skeletal muscle of the rat**
   M. Żendzian-Piotrowska, P. Zabielski, M. Baranowski, J. Górski
5. **The adiponectin level in patients with familial obesity**
   Małgorzata Malczewska-Malec (Poland)
6. **The quest for metabolic syndrome in the nutrigenetics era: beta adrenergic gene polymorphisms and weight lowering therapies**
   Magdalena Szopa (Poland)

**III. Beta-carotene uptake-metabolism**

1. **SNPs as determinants of inter-individual variation in beta-carotene/vitamin A metabolism**
   W. Leung, C. Meplan, J. Hesketh, G. Lietz
2. **Switching between physical and chemical mechanism of ROS quenching by carotenoids**
   J. Fiedor, R. Haessner, H. Scheer, L. Fiedor
3. **Generation and phenotyping of the beta-carotene 15,15'-monooxygenase KO mouse**
   A. Eichinger, R. Goralczyk, A. Wyss
4. **Lutein, zeaxanthin and lipids as risk and protection factors of age-related macular degeneration**
   M. Drobek-Słowik, K. Safranow, K. Jakubowska, M. Rać, D. Karczewicz, D. Chlubek
5. **Susceptibility of lipids from different flax cultivars to peroxidation and its lowering by added antioxidants**
   A. Krasowska, J. Szopa, M. Lukaszewicz

6. **Differences in all-trans beta-carotene (BC) uptake and eccentric cleavage by human endothelial and neoplastic cell lines**
   J. Hartwich, J. Zagajewski, A. Polus, J. Dulińska, T. Sacha, G. Riss, J. Grzybowska, M. Mikołajczyk, P. Laidler, I. Wybrańska, R. Goralczyk, A. B. Skotnicki, A. Dembińska-Kieć
7. **Effects of dietary lycopene on lipid parameters and yolk coloration in Japanese quail**
   L. Bárdos, K. Réthy, Z. Kiss, C. Szabó
8. **Beta-carotene and cigarette smoke: a chemical and biological approach**
   K. Vlismas, F.C.R. Manning, G.M. Lowe
9. **Chemical composition of dried biomass of *Blakeslea trispora***
   E. Kunschikova, V. Narushin, I. Kunschikova, A. Tyurenkov
10. **Profiles of lipids in fruit-bodies of some edible mushrooms**
    K. Grzywnowicz

14:00 –15:00 Lunch break (Zodiak Hall, Św. Anny 12 Str.)

**SESSION III**
**CAROTENOIDS — THE BIOPHYSICAL ASPECTS**
Chairmen: Wilhelm Stahl, Kazimierz Strzałka

**15:00** **Introduction**
Kazimierz Strzałka (Poland)

**15:05** **Carotenoids in nutritional protection**
Wilhelm Stahl (Germany)

**15:35** **Are lycopene and other dietary carotenoids beneficial? Reactions of carotenoids with oxy-radicals and singlet oxygen**
T. George Truscott (United Kingdom)

**16:05** **Carotenoids as modulators of lipid membrane physical properties**
Wiesław Gruszecki, Kazimierz Strzałka (Poland)

**16:35** **Model and cellular in vitro study of antioxidant and photoprotective properties of macular pigments**
Tadeusz Sarna (Poland)

## Sunday, 12 December 2004

## COLLEGIUM NOVUM (AULA)

**THE COMMON SESSION OF "DLARFID"**
**DIETARY CAROTENOIDS AND LIPIDS: LESSON LEARNED FROM EPIDEMIOLOGICAL AND GENETIC STUDIES**
And
**COST B17 ACTION WORKING GROUP 1:**
**GENETIC ASPECTS OF NIDDM, OBESITY, INSULIN RESISTANCE AND AGEING**
Chairmen: Bela Bendlova, Aldona Dembińska-Kieć

**8.15** **Welcome to participants**
Aldona Dembińska-Kieć

**8:30** **Roles of PPAR delta in lipid absorption and metabolism. A new target for the treatment of type 2 diabetes**
Paul A. Grimaldi (France)

| | |
|---|---|
| **9:00** | **A novel method for rapid quantification of free and esterified phytosterols in serum using APPI tandem mass spectrometry**<br>Joachim Thiery (Germany) |
| **9:30** | **Synergistic effects of zeaxanthin and its binding protein in the prevention of lipid membrane oxidation**<br>Prakash B. Bhosale (United States of America) |
| **10:00** | **Induction of PXR-mediated metabolism by $\beta$-carotene**<br>Ralph Rühl (Hungary/Germany) |
| **10.30** | **Association of adipose and red blood cell lipids with severity of dominant Stargardt macular dystrophy**<br>Paul S. Bernstein (United States of America) |
| **11.00** | **The risk polymorphisms of UCP1 and PPARG2 genes in patients with polycystic ovary syndrome**<br>Bela Bendlova (Czech Republic) |
| **11.30–12.15** | **SHORT PRESENTATIONS**<br>**Insulin resistance and the beta cell function in relation to selected DM2 candidate genes**<br>Marketa Vankova (Czech Republic)<br>**The KCNJ11 gene polymorphism E23K in relation to DM2 in Czech population**<br>Daniela Sramkova (Czech Republic)<br>**The additive effect of coexistence more than one "susceptibility gene" alleles on development of obesity and insulin resistance**<br>Iwona Wybrańska, Małgorzata Malczewska-Malec, Aldona Dembińska-Kieć (Poland) |
| | **THE POST-MEETING EVENTS** |
| **12.30–15.00** | **COST 17B MANAGEMENT COMMITTEE MEETING** (Collegium Novum, Sala Senacka) |
| | **WYBRANE PROBLEMY NUTRIGENOMIKI W OCHRONIE ZDROWIA — SESJA STUDIUM MEDYCYNY MOLEKULARNEJ** (Biblioteka Zakładu Biochemii Klinicznej, ul. Kopernika 15a, I piętro) |
| **14.00–14.30** | **Udział aktywatorów PPAR/RXR w różnicowaniu się komórek**<br>Anna Polus, Joanna Grzybowska |
| **14.30–15.00** | **„Kanałopatia" — wpływ diety na genetycznie uwarunkowaną aktywność kanałów jonowych (nadciśnienie; migrena, zaburzenia rytmu; padaczka etc.)**<br>Marek Bodzioch |
| **15.00–15.30** | **Dieta a genetycznie uwarunkowany zespół metaboliczny**<br>Małgorzata Malczewska-Malec |
| **15.30–16.00** | **Genetyczne aspekty chorób złożonych — problemy interpretacji badań polimorfizmu genetycznego i diety**<br>Iwona Wybrańska |
| **16.00–16.30** | **Dieta a hemostaza i angiogeneza**<br>Łukasz Partyka |
| **16.30–17.00** | **Suplementacja witaminami — korzyści i potencjalne zagrożenia**<br>Jadwiga Hartwich |

# Index of the conference materials

CAROTENOIDS AND LIPIDS IN CELL PROLIFERATION AND DIFFERENTIATION (CANCER CELLS)



CARTENOIDS — THE BIOPHYSICAL ASPECT



DIETARY CAROTENOIDS AND LIPIDS: LESSON LEARNED FROM EPIDEMIOLOGICAL AND GENETIC STUDIES/GENETIC ASPECTS OF NIDDIM, OBESITY, INSULIN RESISTANCE AND AGEING

POSTER SESSION

ORAL PRESENTATIONS

## Fatty acid composition of fats as an early determinant of childhood obesity

G. Ailhaud, P. Guesnet
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High-fat diet is known to be associated with fat mass gain. However, there is a lack of a general increase in energy intake as fat among youths despite a striking increase in the prevalence of overweight and obesity in industrial and developing countries. Decreased physical activity and nonexercise activity thermogenesis have been implicated to explain this paradox. Long-term relationship between fatty acid composition of ingested fats and the development of adipose tissue in infants is difficult to assess in contrast to animals. *In vitro*, among natural long-chain fatty acids, dihomo-gamma-linolenic acid (20:3, omega-6) and arachidonic acid (C20:4, omega-6), metabolites of essential linoleic acid (C18:2, omega-6), promote substantially adipogenesis of preadipocytes. Arachidonic acid acts through the prostacyclin signalling pathway which involves the cell surface prostacyclin receptor IP-R. In contrast, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), metabolites of essential alpha-linolenic acid (C18:3, omega-3), are anti-adipogenic and counteract the effect of arachidonic acid. *In vivo*, under isoenergetic conditions, nutritional experiments with linoleic acid-enriched diet and with linoleic acid/$\alpha$-linolenic acid-enriched diet in wild-type and IP-R K.O. mice show the importance of the arachidonic acid/prostacyclin pathway in enhancing adipose tissue development during the gestation/suckling period. Published U.S. data show over the last decades a dramatic and specific increase in the amount of linoleic acid but not in that of alpha-linolenic acid consumed by infants fed breast-milk or formula-milk. Thus it is proposed that the fatty acid composition of ingested fats is an early determinant of childhood obesity, *i.e.* at a time where adipose tissue exhibits a very dynamic phase of its development.

## Challenges to understanding and measuring carotenoid bioavailability

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Carotenoids are an excellent example of where too little understanding of food structure, the complexity of their behaviour during digestion, and the cause of inter-individual response, can lead to misinterpretation of study results. This may lead to confusion in understanding their relevance to health. It is essential that all those utilising the output from bioavailability studies are conversant with the challenges in measuring carotenoid bioavailability, to ensure that model systems for the investigation of functional response are realistic and dietary strategies are soundly based.

The presentation outlines four challenges associated with understanding and measuring carotenoid bioavailability. These are (i) release of carotenoids from the food structure and processing into a potentially absorbable form (bioaccessibility), (ii) passage of carotenoids from gut lumen into the body (absorption), (iii) interpreting plasma response and (iv) inter-individual variation.

In summary, the bioaccessibility of carotenoids is governed by the physical properties of the food matrix, which affects the efficiency of the physical, enzymic and chemical digestion processes.

Carotenoids used as colorants are likely to be relatively better absorbed, because of the form in which they are dispersed in food. The extent of absorption of carotenoid supplements will depend on the size of the dose and proximity of dosing to the consumption of a fat containing meal. The release of carotenoids from food plants occurs only when the plant cell is fractured and this occurs only during food preparation, processing and/or mastication, not during digestion.

Following release from the food matrix the major limiting factor governing the extent of absorption is the solubility in digesta. Carotenoid solubility is a major challenge in the design of cell culture studies. The use of non-physiological carotenoid delivery systems, and the poor understanding of their effect on cell response, is a matter of concern regarding conclusions drawn from cell studies.

Absorption studies are best carried out by measuring the chylomicron carotenoid excursion and modelling this with some measure of chylomicron turnover rate ($t_{1/2}$). In this way inter-individual differences in lipoprotein metabolism can, in part, be taken into account before formulating conclusions on the rate and extent of carotenoid absorption.

## Fatty acids and expression of adipokines

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Adipose tissue has been recognised as the quantitatively most important energy store of the human body for many years, in addition to its functions as mechanical and thermic insulator. In mammals, the adipose organ is localised in several depots including white as well as brown adipose tissues. The largest depots are found subcutaneously and in the abdominal region.

Several secretory proteins are synthesised in adipose tissue including leptin, resistin, adiponectin, tumor necrosis factor (TNF$\alpha$), angiotensinogen, adipsin, acylation-stimulating protein, retinol-binding protein (RBP), interleukin (IL)-1beta, IL-6, IL-8, IL-10, plasminogen activator inhibitor-1 (PAI-1), fasting-induced adipose factor, fibrinogen-angiopoietin-related protein, metallothionein, tissue factor (TF), complement C3, fibronectin, haptoglobin, entactin/nidogen, collagen VI $\alpha$ 3, pigment epithelium-derived factor (PEDF), hippocampal cholinergic neurostimulating peptide (HCNP), neutrophil gelatinase-associated lipocalin (NGAL) and adiponutrin.

Fatty acids may influence the expression of adipokines like leptin, resistin or adiponectin directly by interaction with transcription factors, or indirectly via unknown mechanisms possibly linked to fatty acid oxidation, synthesis or storage. Because fatty acids are the main components of adipose tissue it is of essential interest to clarify the biological effects of different types of fatty acids on the expression of relevant adipokines.

## Structurally different marine oils in health and medicine

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Most of the dietary TAGs are derived from vegetable oils, particularly soybean oil which is very rich in linoleic acid (18:2n-6, LA), which is the precursor of arachidonic acid (20:4n-6, AA). Some vegetable oils like linseed oil and rapeseed oil contain substantial amounts of a-linolenic acid (18:3n-3, ALA). However, the *in vivo* conversion of ALA to eicosapentaenoic acid (20:5n-3, EPA) and particularly to docosahexaenoic acid (22:6n-3, DHA) is limited in humans (Vermunth *et al.* 2000; Pawlosky et al. 2001; de Groot et al. 2004). Thus, the high ratio of n-6/n-3 fatty acids of the Western diet leads to a high ratio of 20:4 n-6/ /20:5n-3 in the blood and tissues. 20:4n-6 in cell membrane phospholipids is the substrate for the synthesis of a range of biologically active eicosanoids. The current Western diet (fat) may therefore promote IBD and other chronic inflammatory diseases like rheumatic disorders by facilitating the production of pro-inflammatory eicosanoids (Gil 2002; Simopoulos 2002). In addition, lipids used in nutritional support of surgical and critically ill patients have been based on soybean oil leading to an increase in the total amount of n-6 fatty acids in cell membranes. There is a view that an excess of n-6 fatty acids should be avoided since this contributes to a state where physiological processes may become dysregulated (Arslan *et al.* 2002; Gil 2002; Simopoulos 2002; Calder 2003). All patients completed treatment with seal oil without relevant protocol violations. No relevant subjective side effects of the seal oil administration were detected. Compared to control subjects, a lower n-3/n-6 ratio was found in rectum biopsies from patients with IBD, but this was normalised after seal oil treatment (Figure 1). Total amount of n-6 fatty acids in serum was significantly reduced ($p = 0.016$) whereas a significant increase ($p = 0.01$) in n-3 fatty acids (20:5n-3, $p = 0.002$ & 22:6n-3, $p = 0.04$) was observed. The total amount of saturated and monounsaturated fatty acids in serum were not changed after seal oil administration. The ratio of n-6 to n-3 in serum was significantly lowered ($p < 0.0001$) by seal oil treatment.

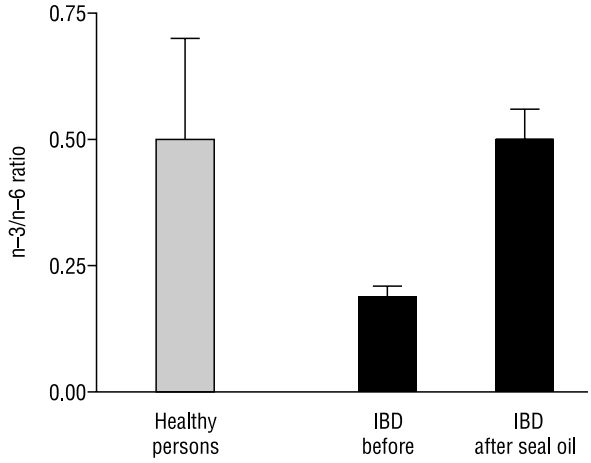


**Figure 1**. n-3/n-6 ratio in control rectum biopsies (n = 20) compared to rectum biopsies from patients with IBD (n = 9) at baseline and after ten days of treatment with seal oil

This study demonstrates that seal oil treatment significantly increases the amount of n-3 PUFAs in serum and rectum biopsies from patients with IBD with a concomitant pain relief. These results are in accordance with a previous pilot study using a short term (ten days) duodenal seal oil treatment to patients with IBD (Arslan et al. 2002). In conclusion, seal oil administration seems to improve the fatty acid profile of the rectum epithelium and possess a pain relieving effect in patients with IBD. Seal oil may prove to become an alternative for nutraceutical as well as additional treatment of IBD and it seems as seal oil represent valuable tool to further study the absorption and clearance of lipids.

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## Mechanisms of genomic and non-genomic actions of carotenoids

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Carotenoids are bioactive dietary compounds that may be able to influence human health. A large proportion of the research to date has focused on the potential role of the carotenoids as dietary antioxidants. However, the true significance of these effects both under normal physiological conditions and conditions of oxidative stress remains unclear. Other mechanisms of action that are independent of their antioxidant properties may be as, or even more, important. One obvious example is that certain members of the carotenoid family can be processed within the body to yield vitamin A, a potent bioactive agent exerting profound effects on processes such as tissue development and disease prevention. But by no means all the observed effects of carotenoids can be explained simply on the basis of pro-vitamin A activity. Overall there is still a considerable degree of uncertainty about the actual mechanisms underlying of various biological effects of carotenoids. The development of functional genomic techniques provides researchers with powerful new approaches that can be used to start to elucidate the relative contributions different mechanisms. However, there are still technical challenges to address. In studies involving human volunteers, the key tissues of interest often are not obtainable in sufficient quantity at serial sampling points or simply not available at all. This makes the use of model systems necessary. If animal models are to be used, very careful selection of the most appropriate model is essential. Human cell culture systems have the advantages of simplicity and the ability to achieve close experimental control. However, most cell culture systems use immortalised or cancer cells that may not always respond in a manner fully representative of normal cells in vivo. Furthermore efficient and representative delivery of the lipophilic carotenoids to cell in culture presents a particular technical challenge. Very careful attention to detail in the design experiments is vital to unravel this complex story.

## Towards a better understanding of carotenoid metabolism in animals

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Animals, in general, cannot synthesize vitamin A *de novo*, but rely on a supply of dietary precursors. All naturally occurring vitamin A derives by enzymatic oxidative cleavage from carotenoids with provitamin A activity. This process follows a universal scheme in animals. To become biologically active, carotenoids must first be absorbed, then delivered to the site of action in the body, and in the case of the provitamin A function, metabolically converted. In the fruit fly *Drosophila* — with vitamin A functions being restricted to the visual system — blind mutants with impairments in this pathway have been isolated. The eye phenotype of *ninaD* flies is characterized by an absence of both carotenoids and retinoids, while in *ninaB* flies only retinoids are missing and carotenoids are highly accumulated. Our analyses revealed that the *ninaD* gene encodes a cell surface receptor rendering carotenoids available cellularly. *NinaB*, a beta, beta-carotene-15,15'-oxygenase, catalyzes the oxidative cleavage of the provitamin A, the key step in vitamin A formation, to give two molecules of retinal. By searching for similar genome sequences in vertebrates, we identified three *ninaB* homologous genes. By loss-of-function experiments, we addressed their roles in vitamin A metabolism during zebrafish development. These analyses revealed that we identified a novel class of carotenoid/retinoid metabolizing enzymes with essential functions in retinoic acid signaling as well as in vision.

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## Morphology of ferret subcutaneous adipose tissue after six-month daily supplementation with oral beta--carotene

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Adipose tissue is an important retinoid depot and retinoids are known to influence white and brown adipocyte metabolism. Identifying nutrients that can affect the biological activity of the adipose organ would be of great medical interest in the light of the current obesity epidemic and related disorders in developed countries. The vast majority of mammal studies of chronic administration of oral beta-carotene have used murine models, while few have employed mammals exhibiting uptake and processing of intestinal beta-carotene similar to those of humans. While rodents transform practically all ingested beta-carotene into retinol, in ferrets, as in humans, part of the beta-carotene is absorbed and released into the circula-

tion intact. We studied the effects of 6-month daily administration of two doses of oral beta-carotene (0.8 mg/kg/day or 3.2 mg//kg/day) on ferret body weight, size of body fat depots, and, using morphological and morphometric methods, on subcutaneous (inguinal) white adipose tissue (WAT). Because of the oral mode of administration, liver, stomach, and small and large intestine were also studied. Control animals received the vehicle. Data show that at the end of treatment the higher dose induced significantly higher body weight compared with controls and significantly higher inguinal fat depot compared with animals treated with the lower dose. In addition, chronic treatment with beta-carotene induced a dose-dependent hypertrophy of white adipocytes and increased neoangiogenesis in subcutaneous WAT in all treated ferrets. Vasculogenesis was independent of adipocyte hypertrophy. We also found focally evident liver steatosis in the ferrets treated with the higher dose of beta-carotene. The other gastrointestinal tract organs studied were not significantly different from those of control animals.

## Gene regulation by beta-carotene in ferrets

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The ferret have been proposed as useful model to study human beta-carotene absorption and cleavage as these animals also absorb and release intact beta-carotene from the enterocyte. One of the interest for the study of beta-carotene effects on these animals come from epidemiological studies showing that people that eat more fruits and vegetables (which are rich in carotenoids) and people having higher serum beta-carotene levels have a lower risk of cancer, particularly lung cancer. However, the two main human intervention studies using moderate daily doses of beta-carotene supplementation (20 mg in the ATBC trial; 30 mg in CARET trial) revealed an increased risk of lung cancer among smokers and asbestos workers. The knowledge on beta-carotene metabolism in both humans and ferrets is scarce and the vast majority of studies on mammalian beta-carotene have used rodents or other laboratory small mammals, this species not absorbing beta-carotene as such care.

Previous studies carried out in the ferret have reported that the administration of a pharmacological dose (2.4 mg/kg body weight/day) of beta-carotene (SIGMA) lead to squamous metaplasia and increases the expression of some proto-oncogenes. Conversely, a physiological dose of beta-carotene (0.43 mg/kg body weight/day) did not show these effects, but even provided mild protection again squamous metaplasia in smoke-exposed ferrets. We treated ferrets with two oral doses (0.8 and 3.2 mg/kg body weight/day) of beta-carotene for 6 months; beta-carotene used was supplied from *DSM Nutritional Products Ltd.* as a water soluble formulation (beadlets) containing 0.1% beta-carotene crystalline, 0.015% DL-alpha-tocopherol, 0.04% ascorbyl palmitate, 0.04% corn oil, 0.15% fish gelatine, 0.34% sucrose and 0.315% corn starch. We did not find apparently detrimental effects of beta-carotene on lung cell proliferation. These seemingly paradoxical results suggest that, in addition to the dose, the formulation (e.g. the presence of alpha-tocopherol or other antioxidant compounds) may be important in determining the pro- or anti-carcinogenic effects of beta-carotene.

We also found that ferrets treated with the high dose of beta-carotene gained more weight than control animals and had a higher size of the inguinal subcutaneous fat depot. More studies are being carried out to elucidate the mechanistic basis for these observations.

## Gene expression profiling identifies retinoids as potent inducers of macrophage lipid efflux

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Vitamin A and its naturally occuring derivatives 9-cis retinoic acid (9-cis RA) and all-trans retinoic acid (ATRA) exert a variety of biological effects including immunomodulation, growth, differentiation, and apoptosis of normal and neoblastic cells. In order to directly study the effects of these retinoids on macrophage gene expression and lipid metabolism, primary human monocytes and *in-vitro* differentiated macrophages were stimulated with $\beta$-carotene, 9-cis RA, and ATRA and global gene expression profiles were analyzed by Affymetrix DNA-microarrays and differentially regulated genes were verified by quantitative TaqMan RT-PCR. Among others, we have identified a strong upregulation of a cluster of genes involved in cholesterol metabolism including apolipoproteins (apoC-I, apoC-II, apoC-IV, apoE), the scavenger receptor CD36, steroid-27-hydroxylase (CYP27A1), liver-X-receptor $\alpha$ (LXR$\alpha$), and ATP-binding cassette transporters A1 (ABCA1) and G1 (ABCG1). The induction of these genes could be also confirmed in the human THP-1 monocytic cell model. Interestingly, the induction of these genes was most effective with 9-cis RA and was independent of monocytic differentiation and lipid loading. Since the CYP27A1 gene displayed the strongest upregulation on the mRNA level, we cloned various deletion contructs of the promoter region and analyzed the response to

retinoids in macrophages. Thereby, a novel retinoid acid-responsive element could be located within 191bp of the proximal CYP27A1 promoter. To further assess the functional consequences of retinoid receptor action, we carried out phospholipid and cholesterol efflux assays in stimulated macrophages. A strong induction of apoA-I-dependent lipid efflux, without prior loading of cells with modified lipoprotein-derived or free cholesterol could be observed. This clearly implies that the strong positive effects of retinoids on the expression of key genes in macrophage cholesterol metabolism equally translates into biological functions reflected by a strong increase in specific lipid efflux.

## Vitamin A as a regulator of adipogenesis and adipocyte metabolism-derived medical complications

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Beyond their classical nutritional value, nutrients affect many fundamental biological processes through modification of gene expression and function. An emerging example is the involvement of vitamin A in the control of biological aspects regulating the level and functioning of body fat reserves.

Retinoic acid (RA), the carboxylic acid form of vitamin A, influences adipocyte differentiation and survival, with high doses inhibiting and low doses promoting adipogenesis of preadipose cells in culture. In addition, RA signals transcriptional activation of the genes encoding uncoupling proteins (UCPs), which are mitochondrial proteins involved in energy dissipation and fatty acid metabolism. In rodents, acute administration of RA results in reduced body weight and adiposity in the absence of changes of food intake, increased thermogenic capacity in brown adipose tissue and muscle, reduced adipogenic and lipogenic capacity in adipose tissues, and appearance of metabolic features of brown adipocytes (which are cells specialized in inefficient fuel oxidation) in white adipose tissue depots. Chronic dietary vitamin A supplementation also increases thermogenic potential of brown fat and muscle, and appears to confer some resistance to high-fat diet induced obesity in rodents. A poor vitamin A status, on the other hand, favors in rodents an increment of adiposity and there are some studies linking a low dietary intake of vitamin A with a high incidence of obesity in human populations. RA also has important, direct effects on the expression of adipocyte-derived protein signals involved in the control of energy balance, adipose tissue development and insulin sensitivity, such as leptin and resistin, inhibiting the expression of both.

Knowledge of the impact of vitamin A derivatives in the regulation of adiposity, insulin sensitivity and energy expenditure may serve to define new directions for the development of nutritional and pharmacological strategies to efficiently combat obesity and type II diabetes, two closely related metabolic diseases with an increasing and alarming incidence worldwide.

## Transgenic embryonic stem cells for basic research and clinical application

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Because of their ability to reproduce the embryological differentiation of nearly all different cellular phenotypes, embryonic stem (ES) cells represent an ideal tool to to study processes of embryogenesis under *in vitro* conditions, in particular the signalling cascades and genes involved in the functional development (functional genomics) as well as to provide a new source for cellular replacement therapy. We have cultivated ES cells in three dimensional cell aggregates, where they differentiate into derivatives of all three germ layers.

1. Embryonic vascularization comprises different processes such as proliferation, migration, differentiation and tube-formation of endothelial cells. To date little is known about morphogenetic changes of endothelial cells and the molecular mechanisms involved occurring during early stages of embryonic development. We have therefore established stably transfected mouse ES cell lines, where the endothelial-specific platelet endothelial cell adhesion molecule (PECAM) promoter drives the expression of the *live* reporter enhanced green fluorescent protein (EGFP). This approach enables investigation of morphogenetic changes and related signalling cascades in endothelial cells during early embryonic development. Morphogenetic changes of endothelial cells in the presence of key regulatory molecules fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) are monitored employing time lapse microscopy. Three days post plating EGFP positive cells are detected in the ES cell aggregates. At first, clusters of angioblasts are predominant which later develop into elongated network-like structures. VEGF induces proliferation and pronounced sprouting angioblasts whereas FGF leads to stabilization of preformed endothelial structures. We conclude that the ES cell system in combination with endothelial-specific EGFP expression is a valid tool to investigate early events of vascular differentiation *in vitro*. VEGF plays an important role for early vessel formation whereas FGF is crucial for endothelial survival. Because of their ability to promote capillarization and angiogenesis ES cells may be usefull for recapillarization after cardiac infarction.

2. This potential is paralleled by their ability to generate cardiomyocytes *in vitro* for tissue repair. Cardiomyocytes differentiated from ES cells were injected into the cryoinfarcted left ventricular wall of adult wild type mice. Immunological cross reactions were avoided by using the same inbred mouse strain. To allow identification of the transplanted cells transgenic ES cells were used carrying an IRES vector with two cloning sites for EGFP and an antibiotics resistance for selective selection both under the $\alpha$-MHC promoter. EGFP positive transplanted cardiomyocytes could be easily detected in the native heart at different intervals after operation. The cells were found to engraft and differentiate into adult-like cardiomyocytes as confirmed by cross striation after immunostaining with $\alpha$-actinin. These data were corroborated by patch clamp experiments on isolated EGFP positive cardiomyocytes at different time points after operation. The transplanted cells displayed ventricular action potentials and $\beta$-adrenergic — as well as muscarinic regulation. When survival was investigated in a large colony of transplanted- (n = 99) and control mice (n = 36) where NaCl instead of transgenic cardiomyocytes were injected, the control group had an almost double mortality rate. Our data show engraftment and differentiation of embryonic cardiomyocytes after transplantation into cryoinfarcted areas of heart.

3. Before a clinical use of human ES cells for therapeutic trials in humans two major prerequisites must be fulfilled: (i) they must be safe, i.e. the development of tumours because of the high proliferative potential must be omitted and (ii) the rejection of the transplanted cells must be prevented. For the criterion (i) the technology of "lineage selection" has been developed, strategies in order to only allow the needed cell to differentiate from ES cells but all other cells are prevented to survive. This can be obtained by modifying the culture conditions and/or adding a combination of various growth factors and signalling molecules which preferentially supports the growth of a specific cell type but prevents the development of other types. However, up to now, the transgenic drug selection approach has proven to be the most specific and effective for reliable purification of ES cell derived cells. For example, IRES-vectors encoding EGFP and the puromycin resistance gene under specific promoters are used. Application of puromycin (optimal time point will be seen from the EGFP expression) will eliminate all other cells except those containing the resistance gene. In our experiments using cardiac specific promoters we found an extremely efficient purification of over 99%. For the criterion (ii) several suggestions have been made: the most simple one is of course the development of a stem cell bank containing 1000 or more ES cell lines with different immunological determinants (MHC surface complexes, HLA system). Alternatively it is suggested to use the so called "therapeutic cloning", i.e. the exchange of the haploid nucleus of a donor oocyte by that of the patient. It has been demonstrated that the oocyte with the new nucleus develops to a normal blastocyst from where a new patient identical ES cell line can be prepared from the inner cell mass. In order to prevent the high number of donor oocytes it may be also possible to directly differentiate oocytes from ES cells.

## PPAR$\gamma$ in the control of brown adipocyte differentiation

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The effects of fatty acids and retinoic acid (carotene) on brown adipose tissue differentiation are mediated by the transcription factors PPAR$\gamma$ and PPAR$\alpha$ in combination with RXR. There is good support for the idea that stimulated PPAR$\gamma$ promotes adipogenesis also in brown adipose tissue. However, the issue is more complex concerning the full differentiation to the brown adipocyte phenotype, particularly the expression of the brown-fat-specific marker UCP1. The effect of norepinephrine on PPAR$\gamma$ gene expression, at least in-vitro, is negative, PPAR$\gamma$-ablated brown adipose tissue can express UCP1, and PGC1-$\alpha$ may coactivate other transcription factors (including PPAR$\alpha$); thus, the significance of PPAR$\gamma$ for the physiological control of UCP1 gene expression is not settled. However, importantly, the effects of PPAR$\gamma$ agonists demonstrate the existence of a pathway for brown adipose tissue recruitment that is not dependent on chronic adrenergic stimulation and may be active in recruitment conditions such as prenatal and prehibernation recruitment. The ability of chronic PPAR$\gamma$ agonist treatment to promote the occurrence of brown-fat-features in white adipose tissue-like depots implies a role in anti-obesity treatment, but this will only be effective if the extra thermogenic capacity is activated by adrenergic stimulation.

## Regulation of adipocyte differentiation and function by polyunsaturated fatty acids

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A diet enriched in PUFAs, in particular of the n-3 family, decreases adipose tissue mass and suppresses development of obesity in rodents. Although several nuclear hormone receptors are identified as PUFAtargets, the precise underlying mechanism behind this effect is still not elucidated. Here we re-

view research aimed at elucidating molecular mechanisms governing the effects of PUFAs on differentiation and function of white fat cells with special emphasis on dietary PUFAs as signaling molecules.

## Molecular mechanisms controlling white versus brown adipocyte differentiation

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In response to cues that still remain enigmatic, mesenchymal stem cells give rise to adipocyte precursor cells, which subsequently differentiate into one of two major cell populations with different physiological roles, white and brown adipocytes. Two major regulators of the mammalian cell cycle, the retinoblastoma protein (pRB) and p53 have been implicated in the control of adipocyte differentiation. Initially, it was reported that pRB was required for adipocyte differentiation, whereas circumstantial evidence linked p53-deficiency to brown adipocyte differentiation. We have now demonstrated that the situation is much more complex in that pRB promotes differentiation of white adipocytes, but inhibits the formation of brown adipocytes. In contrast, p53 appears to be a general inhibitor af adipocyte differentiation, and down-regulation of p53 expression seems to be an integral part of the differentiation program leading to either white or brown adipocytes.

## Umbilical cord progenitor cell differentiation in the presence of PPAR-gamma and RAR/RXR activators

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Peroxisome proliferation-activated receptor gamma (PPAR-gamma) stimulated by fatty acids, its derivates as well as exogenous compounds (e.g. glitazones) heterodimerizing with the carotenoid/retinoid-activated RAR/RXR transcription factors are important regulators of cell fate. Progenitor stem cells persist in adult life, and contribute in the tissue repair such as in neovasculogenesis. Markers of circulating early endothelial progenitors cells (EPC) are (in between): CD34, AC133, PECAM-1/CD31, VE-cadherin and flk1 (VEGF-R2). Such cells leaving bone marrow are under influence of circulating in blood lipoproteins with their lipids and lipid-soluble compounds including retinoids.

Aim of the study was to analyze the human endothelial (HUVEC) and umbilical cord (UPC) differentiation under influence of lipoprotein PPAR/RAR/(RXR) activators

Material and methods: CD34/AC133 were isolated from human cord blood using magnetic microbits (Milteny Biotech). Primary cultures of HUVEC were isolated from human umbilical vein using collagenase digestion method. Cells were grown in EBM medium with supplement and antibiotics. Influence of PPARs activators (palmitic, linoleic , arachidonic acids, lysophosphatidilcholine (oxPAPC, SPP-1) or $\beta$-carotene on the gene expression after 24 hour incubation was estimated by real-time PCR (Opticon) or by oligonucleotide chips (Affymetrix) analysis. The changes in certain protein synthesis was proved by flow-cytometry. To determine the effect of factors on cell proliferation, the rate of DNA synthesis was estimated by measuring BrdU incorporation. Influence of above compounds on first step of angiogenesis — the tube formation was investigated in the 3D in vitro Matrigel model assay. Stromal Derived Factor (SDF) or VEGF- induced chemotaxis was performed and measured by using BD Falcon Fluoro Bloc System (Becton Dickinson).

Results: On the contrary to VEGF, bFGF or leptin, the PPAR-$\gamma$ activators and $\beta$-carotene in non-toxic concentrations did not significantly influence the proliferation and the tube formation in the tubulogenesis model in vitro. In spite of not significant influence of modified lipoproteins (ox LDL, oxPAPC) on HUVEC or EPC migration, the VEGF or SDF-induced chemotaxis was significantly modified. $\beta$-carotene alone stimulated chemotaxis of the EPC. Microarray and real-time PCR analysis indicated that beta-carotene activated the gene expression connected with cell differentiation, cell cycle, adhesion, cell-cell signalling chemotaxis, apoptosis as well as with induction of cyt P450 pathway of xenobiotic catabolism. The expression of the EGF-1 transcription factor seems to play important role as the common regulator of above processes.

Conclusion: Our results confirm the influence of circulating lipoprotein PPAR/RAR/RXR agonists on the priming of progenitor cells in early, invasive stages of new vessels formation what may by the undesirable side-effect of the supportive therapy with bone marrow progenitors such as neovasculogenesis associating atherosclerosis or diabetic retinopathy.

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## Effects of β-carotene and lycopene in cells exposed to cigarette smoke condensate: modulation of redox sensitive molecular pathways involved in cell growth

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Human intervention trials suggested that β-carotene supplementation results in an increased lung cancer incidence in smokers, but not in non smokers. Since it is known that cigarette smoke contains a large amount of free radicals and that its metabolism further contributes to their formation, we studied the relationship existing between cell growth and redox status in Mv1Lu lung, MCF-7 mammary, DU145 prostate and LS-174 colon cancer cells and immortalized RAT-1 fibroblasts cells exposed to cigarette smoke condensate (tar) and β-carotene or lycopene. We found that the association of tar and β-carotene was able to: 1) increase cell number and the percentage of cells in the S-phase through an increase of cyclin D1 and a decrease in the levels of p53 and p21 with respect to tar alone; 2) inhibit apoptosis through changes in Bax protein; 3) exacerbate DNA oxidative damage, measured as 8-OHdG formation; 4) increase the expression of Cox-2, a protein involved in the production of reactive oxygen species and a suitable marker of smoke-related carcinogenesis; 5) modulate the expression of some redox-sensitive enzymes, such as heme oxygenase-1 (and its repressor gene Bach1), NADPH quinone oxidoreductase, cytochrome P450 reductase. Such effects were found at low concentrations of the carotenoid, ranging from 0.75 to 4.0 μM, and were not observed when cells were exposed to β-carotene alone. Surprisingly, opposite effects on these parameters were obtained using lycopene in association with tar in the same range of β-carotene concentrations. In all the cells analysed, lycopene acted as an antioxidant, inhibiting DNA oxidative damage, cell growth and the expression of Cox-2. Both β-carotene and lycopene were consumed by smoke. However, while the oxidation of lycopene reduced its antioxidant and growth-inhibitory effects, the oxidation of β-carotene enhanced its prooxidant and growth-promoting activity, strongly suggesting that oxidative metabolites of β-carotene may be implicated in the control of cell growth. These findings support the hypothesis that a redox mechanism may be implicated in the regulation of cell growth by carotenoids and suggest a possible explanation for the detrimental effects of an association of smoke and β-carotene found in human trials.

## β-carotene-induced changes in RARβ isoform expression pattern do not influence lung adenoma multiplicity in the NNK-initiated A/J mouse model

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Dietary beta-carotene (bc) has been associated with decreased lung cancer risk, however intervention studies in smokers reporting increased lung tumor rates after high long-term bc supplementation. To establish reasons for these conflicting results, we studied the influence of bc on tobacco smoke carcinogen-induced lung cancer development in the A/J-mouse using 4-(N-Methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) as initiator, and lung adenoma multiplicity as functional endpoint. Gene regulation of the putative tumor suppressor RARbeta was analyzed by quantitative Real-time PCR for its relevance to predict the endpoint lung cancer.

A/J-mice achieved plasma bc levels of up to 3 micromol/L within 4 weeks, and up to 6 micromol/L after 6 months of supplementation on a diet modified to enhance bc absorption. Despite high lung bc concentrations of up to 6 micromol/l/kg, tumor multiplicity was not significantly affected by the bc treatment, neither in carcinogen-initiated or in uninitiated mice, and irrespective of dose and time point of treatment during cancer formation. Tumor multiplicity was not correlated with bc plasma levels in NNK-treated animals. All RARbeta isoforms were significantly suppressed in lungs of NNK-treated animals irrespective of bc treatment. However, the number of tumors per mouse did not correlate with the RARbeta-isoform expression levels. Bc alone mildly but significantly increased RARbeta1 and RARbeta2 and 4 after 3 months supplementation. This induction persisted until 6 months for RARbeta2 and 4. In conclusion, bc-induced change in RARbeta-isoform expression pattern was not predictive for tumor multiplicity, but rather indicative for an intact bc metabolism and persistent sensitivity to retinoic acid in the mice.

## The effect of $\beta$-carotene in colon and lung epithelial cells

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We have studied the effect of $\beta$-carotene on gene expression in intestinal and lung epithelial cells. First *in vitro* studies were started in human cell lines. To make a selection of relevant cell lines and exposure times, intracellular $\beta$-carotene and metabolite levels were measured in a panel of human intestinal and lung cell lines that were exposed to 1 $\mu$M $\beta$-carotene during 2, 6, 30, 54 hours and 3 weeks. The panel included three colorectal carcinoma cell lines (Caco-2, HT-29, HT29D4), an SV40 transformed colon cell line (CCD 841 CoTr), an intestinal carcinoma cell line (HuTu 80), three lung carcinoma cell lines (NCI-H661, NCI-H292, NCI-H460) and an SV40 transformed lung cell line (BEAS-2B). A single run HPLC method was used for this purpose, which allowed simultaneous measurement of intracellular carotenoid and retinoid levels. The intracellular $\beta$-carotene levels varied between the cell lines. The three colorectal carcinoma cell lines showed low levels of $\beta$-carotene which linearly increased during the whole exposure period of 3 weeks. The remaining cell lines had much higher intracellular $\beta$-carotene levels, which stabilized after 54 hours of exposure. Also metabolite profiles appeared to diverge between the various cell lines.

Two lung cell lines were chosen for further gene expression studies using cDNA microarrays, the lung carcinoma cell line NCI-H661 and the SV40 transformed lung cell line BEAS-2B. The cell lines were exposed to 1 $\mu$M $\beta$-carotene during 78 hours and RNA samples were hybridized to a 3K human cDNA array to find up- and down-regulated genes. The changes in gene expression induced by the $\beta$-carotene exposure appeared to be small (maximum 2.4 fold for NCI-H661 and 2. 1 fold for BEAS-2B). For both cell lines the number of differentially expressed genes was small. NCI-H661 and BEAS-2B showed 16 and 20 differentially expressed genes, respectively, more than 1.5 fold up- or down-regulated ($p < 0.05$, $n = 5$). Different genes were found in the two cell lines. Principle Component Analysis (PCA) showed that the basal gene expression differences between the cell lines were bigger than the gene expression changes induced by beta carotene, confirming differences between the cell lines. The microarray results could be confirmed by Q-PCR. In addition to *in vitro* studies, gene expression studies were performed in a ferret model in collaboration with Palou et al.

## The effect of beta-carotene and fatty acids on proliferation and apoptosis of human melanoma and prostate cancer cells

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High doses of beta-carotene and other carotenoids have been recognized for their anti-cancer activity, either because of antioxidant potential or due to their possible conversion to retinoic acids. Cellular responses to retinoids are generally mediated by two families of nuclear receptors RARs and RXRs. Fatty acids are common components of regular diet and also do not remain without effect on a development and progression of melanoma and prostate cancer. Several studies suggested that essential fatty acid and their metabolites can induce apoptotic death of cell and this action seem to depend on their ability to augment free radical generation and lipid peroxidation. It has been proposed that the $\omega$-6 fatty acids increase the rate of tumor growth. The essential fatty acids, linoleic acid and arachidonic acid (AA), and the AA metabolite $PGE_2$ stimulate tumor growth while oleic acid and the $\omega$-3 fatty acid, eicosapentaenoic acid inhibited growth in cancer cell line. Therefore, we studied the effect of physiological concentration of arachidonic acid and beta carotene on cell growth and gene expression in human melanoma cell lines A 375 and WM 35 as well as human prostate cancer LNCaP and PC-3.

Material and methods: Studies were carried on human cancer cell lines: A375, WM-35 — melanoma and LNCaP (androgen dependent), PC-3 (androgen-independent) prostate cancer. The cells were stimulated by 10 $\mu$M, 20 $\mu$M BC and/or by linoleic, arachidonic acid (0.5; 2 and 5 $\mu$M). The proliferation cells was determined by ELISA BrdU and Crystal Violet Test and cytotoxicity using LDH Test. The expression of genes was studied using RT-PCR, real-time PCR and Microarray analysis (Affymetrix HG-U133A; 22 000 genes). Protein expression was monitored by Western blot.

Results: BC+AA decreased proliferation of LNCaP prostate cancer cells(~25–35%) as well as melanoma A 375 cells (~25%). In case of LNCaP prostate cancer cells, microarray analysis reviled that the expression of number of genes involved in replication, transcription and translation processes, steroid metabolism, cholesterol and eicosanoid metabolism was affected. We also observed decreased expression of c-myc, MAD, MAX. and the changes in expression of the genes which control the transition from G1 to S phase of cell cycle — cytokines and CDK (up regulated with BC +AA), Rb and p107 (up regulated with AA + BC) and E2F (down regulated with BC + + AA). Similarly, microarray analyses indicated that in melanoma

BC + AA might affect cell growth also by modulation of gene expression that control G1/S checkpoint (cyclins and their catalytic subunits and gene involved in replication). This analysis showed no direct correlation with the expression of cdk2 and RB1 on protein levels. The data suggested, that BC + AA induced cell cycle delay in G1 phase most likely by changes in physiological activity of proteins involved in cell cycle progression.

1. The effect of arachidonic acid and beta carotene on human melanoma cell growth. D. Gil[1], W. Placha[1], J. Dulińska[1], A. Kieć--Dembińska[2], G. Schmitz[3], P. Laidler[1]

2. Prostate cancer and supplementation with fatty acid and beta carotene: cell cycle regulation. J. Dulińska[1], D. Gil[1], W. Placha[1], A. Dembińska-Kieć[2], G. Schmitz[3] P. Laidler[1]

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## Role of lycopene and tomato products in prostate health

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Epidemiological evidence associating the decreased risk of prostate cancer with frequent consumption of tomato products inspired us to conduct a small intervention trial among patients diagnosed with prostate adenocarcinoma. Tomato sauce pasta was consumed daily for three weeks before their scheduled prostatectomy and biomarkers of tomato intake, prostate cancer progression and oxidative DNA damage were followed in blood and the available prostate tissue. The whole food intervention was so well accepted by the subjects, that the blood lycopene (the primary carotenoid in tomatoes responsible for their red color) doubled and the prostate lycopene concentration tripled during this short period. Oxidative DNA damage in leukocytes and prostate tissues was significantly diminished, the latter mainly in the tumor cell nuclei, possibly due to the antioxidant properties of lycopene. Quite surprising was the decrease in blood prostate-specific antigen, which was explained by the increase in apoptotic death of prostate cells, especially in carcinoma regions. Prostate cancer cell cultures (LNCaP) were also sensitive to lycopene in growth medium, which caused an increased apoptosis and arrested the cell cycle. A possible explanation of these promising results may reside in lycopene effects on the genes governing the androgen stimulation of prostate growth, cytokines and on the enzymes producing reactive oxygen species, all of which were recently discovered by nutrigenomic techniques. Other phytochemicals in tomato may act in synergy with lycopene to potentiate protective effects and to help in the maintenance of prostate health.

## The *in vitro* effect of $\beta$-carotene and arachidonic acid on cytotoxicity, proliferative potential, differentiation and apoptosis on the acute myeloid leukemia cells

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Background: There is considerable evidence from cell culture and animal models experiments, that antioxidant vitamins and related micronutrients are able to influence the carcinogenic process. beta-carotene (BC) appears to have the strongest protective effect in lowering the risk of cancer. Beta-carotene can modulate molecular pathways involved in the cell cycle progression and enhance the apoptosis. Several lines of evidence indicate that arachidonic acid (AA) or its metabolites are involved in modulating growth and survival of hematopoietic cells. It is suggested that the regulation of differentiation, proliferation and apoptosis of healthy cell differ significantly from the cancer cell line fate. In recent years much interest has been devoted to the carotenoids as factors playing essential role in regulation of cellular mechanisms in normal and neoplasmatic cells.

Aim of the study: This study was aimed to elucidate some aspects of the beta-carotene and its derivatives such as proliferation, differentiation and apoptosis of acute myeloid cells (AML).

Material and methods: Hematopoietic cells were obtained from bone marrow biopsy of 8 de novo AML patients during diagnostic procedures. Cells ($1 \times 10^6$) were cultured in the presence of BC (1, 3, microM) and AA (10 microM) for 24, 48, 72 and 96 hours. The cytotoxicity of respective test substances was measured using CytoTox 96; Non-Radioactive Cytotoxicity Assay (Promega, Madison, USA). The effect of beta-carotene on cell cycle progression and/or apoptosis in leukemic cells was performed using the Cell Proliferation ELISA BrdU colorimetric test (Roche, Mannheim, Germany). The reaction product was quantified by measuring the absorbance at respective wavelength by a scanning multiwell spectrophotometer. The flow cytometry method was used to check the differentiation status of cells before and after all experiments using murine anti-human antibodies: CD4, CD11b, CD13, CD14, CD15, CD33, CD34, CD45, CD117 and HLA-DR (Becton Dickinson, Mountain View, USA). A multicolor analysis of cell surface molecules was measured using flow cytometer FACSCalibur (Becton Dickinson, Mountain View, USA).

Detection of the apoptotic cells was performed using Annexin V Assay and TUNEL method (Pharmingen, San Diego, USA) with FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA). After 24 up to 96 h incubation with BC and AA, colony forming unit-leukaemic (CFU-L) were assayed in methylcellulose serum-free medium with growth factors: stem cell factor, IL-3, IL-6, G-CSF and GM-CSF (Methocult, StemCell, Canada) in 14 days in vitro cultures. All cultures were performed in duplicate and colony counts were performed on day 14.

Results: We did not observe the cytotoxic effect neither of 1 and 3 microM concentration of beta-carotene nor of 10 microM concentration of arachidonic acid. Cytotoxicity of beta-carotene was seen after incubation of studied cells with 10 microM concentration for 24 hours. For further experiments non-toxic concentration of BC or AA was chosen (3 and 10 microM respectively). The 24, 48, 72 and 96 hour coincubation of AML cells with BC and AA did not reveal any influence on the cellular proliferation and the CFU-L number in the clonogenic assay. Even 96 hours treatment of AML cells with BC and AA did not reveal any influence on the cellular diferentiation. The induction of cellular apoptosis by beta-carotene was not seen in all AML patients. Conclusion: In this study we demonstrated that beta-carotene did not affect the proliferation, differentiation and apoptosis of AML cells. The interesting observation is that the achievable in vivo concentration of beta--carotene (10 microM) is cytotoxic for hematopoietic cells in vitro.

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## Cancer prevention by retinoids and carotenoids: independent action on a common target

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Virtually all human tumors are deficient in gap junctional communication (GJC) and restoration of GJC by forced expression of connexins reduces indices of neoplasia. Expression of connexin 43 (C × 43) is upregulated by cancer-preventive retinoids and carotenoids which correlates with suppression of carcinogen-induced transformation in 10T1/2 cells. However, the molecular mechanism for upregulated expression is poorly understood. The retinoic acid receptor antagonist, Ro 41-5253, suppressed retinoid-induced C × 43 protein expression in 10T1/2 cells and induction of a C × 43 luciferase reporter construct in F9 cells, but did not suppress protein expression or reporter activity induced by the non-pro-vitamin A carotenoid astaxanthin. In contrast, C × 43 induction by astaxanthin, but not by a RAR-specific retinoid, was inhibited by GW9662, a PPAR-$\gamma$ antagonist. Neither compound required protein synthesis for induction of C × 43 mRNA, nor was the 5.0 hour half-life of C × 43 mRNA altered, indicating direct transcriptional activation. The responsive region was found within –158 bp and +209 bp of the transcription start site. Site directed mutagenesis of a GC-box in this region increased basal levels of transcription and loss of retinoid responsiveness. Simultaneous treatment with a retinoid and $\beta$-carotene or astaxanthin resulted in supra-additive C × 43 expression, again indicating separate mechanisms of gene regulation.

## Carotenoids in nutritional protection

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Carotenoids comprise a class of natural fat-soluble pigments which are found in numerous fruit and vegetables. Following a diet rich in carotenoids has been epidemiologically correlated with a lower risk for several diseases. The antioxidant activity of carotenoids and biochemical properties influencing signaling pathways have been discussed as basic mechanisms of prevention. Conflicting data from intervention studies with $\beta$-carotene to prevent cancers and cardio-vascular disorders have challenged the concept. However, there is still convincing evidence that carotenoids are important components of the antioxidant network. Photooxidative damage is suggested to be involved in the pathobiochemistry of several diseases affecting the skin and the eye and carotenoids may protect light-exposed tissues. Lutein and zeaxanthin are the predominant carotenoids of the retina and are considered to act as photoprotectants preventing retinal degeneration. The unique distribution, localization and high levels of both carotenoids within the macula lutea as well as their physicochemical properties make them suitable candidates for photoprotection. Beta-carotene is used as an oral sun protectant for the prevention of sunburn and has been shown to be effective either alone or in combination with other carotenoids or antioxidant vitamins. Protective effects are also achieved with a diet rich in lycopene. Carotenoids are important components of a healthy diet and contribute within the network of vitamins and phytochemicals to the maintainance of health.

## Are lycopene and other dietary carotenoids beneficial? Reactions of carotenoids with oxy-radicals and singlet oxygen

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Carotenoids play diverse roles in biology and medicine. Both the quenching of singlet oxygen (energy transfer) and interaction with oxy-radicals (electron transfer, H-atom transfer and addition reactions) are key processes in understanding many of these roles and especially their roles as dietary supplements. Previous work in "simple" solvents will be reviewed and new results in cell membrane models will be presented and discussed in terms of carotenoid structure, and their influence on the properties of the lipid membrane. The formation of aggregates by polar carotenoids is also proposed to be of significance in their ability to quench singlet oxygen.

The possible consequences of using lycopene and other carotenoids as dietary supplements will be discussed.

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## Carotenoids as modulators of lipid membrane physical properties

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Carotenoids are a group of pigments present both in plant and animal kingdom, that play several important physiological functions. The protection against active oxygen species realised via quenching of excited states of photosensitising molecules, quenching of singlet oxygen and scavenging of free radicals is one of the main biological functions of carotenoids. Several recent research indicate that protection of biomembranes against oxidative damage can be also realised via modification of physical properties of the lipid phase of the membranes. This work presents overview of research on an effect of carotenoids on structural and dynamic properties of lipid membranes carried out with application of different techniques such as Electron Paramagnetic Resonance, Nuclear Magnetic Resonance, Differential Scanning Calorimetry, X-Ray diffractometry, monomolecular layer technique and other techniques. It appears that in most cases polar carotenoids span lipid bilayer and have their polar groups anchored in the opposite polar zones of the membrane. Owing to the van der Waals interactions of rigid rod-like molecules of carotenoid and acyl chains of lipids pigment molecules rigidify the fluid phase of the membranes and limit oxygen penetration to the hydrophobic membrane core susceptible to oxidative degradation.

## Model and cellular *in vitro* study of antioxidant and photoprotective properties of macular pigments

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A growing body of epidemiological data suggests that the amount of lutein and zeaxanthin, the two most prominent pigments of the macula lutea, inversely correlates with the incidence of age-related macular degeneration (AMD), the major cause of blindness in people over 60 in developed Western countries. Although the exact mechanism of this phenomenon remains to unknown, experimental data obtained in model systems of different complexity indicate the role of macular pigments in protecting the retina against oxidative stress that may be involved in the pathogenesis of AMD. In this study, we examined antioxidant efficiency of zeaxanthin in protecting liposomal membranes against oxidative damage induced by aerobic photoexcitation of Rose Bengal and ARPE-19 cells against photodynamic damage mediated by Merocyanine 540. Progress of photosensitized peroxidation of lipids in multilayer liposomes made of DMPC/POPC and cholesterol, was monitored by EPR-oximetry and HPLC-EC(Hg) determination of lipid hydroperoxides. Cell survival was determined by MTT assay and cell membrane damage was assessed by HPLC-EC(Hg) determination of cholesterol hydroperoxides using the endogenous cholesterol as a mechanistic reporter molecule. The efficiency of zeaxanthin, vitamin E and vitamin C in protecting against singlet oxygen and free radicals, generated by photosensitized oxidation reactions, was determined by measuring singlet oxygen- or free radical-specific products of cholesterol oxidation. We found that in model membranes, zeaxanthin was a very efficient quencher of singlet oxygen. Although this carotenoid was rapidly consumed due to interaction with free radicals, combination of zeaxanthin with vitamin E or vitamin C exerted a synergistic protection against both singlet oxygen and free radical dependent oxidation of model and cellular membranes. Cells with added antioxidants, exhibited increased resistance to photodynamic damage and accumulated less

lipid hydroperoxides. The data indicate the importance of the antioxidant interaction in protection against oxidative damage to cell membranes.

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## Roles of PPAR delta in lipid absorption and metabolism: a new target for the treatment of type 2 diabetes

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Peroxisome proliferator-activated receptors (PPARs) are lipid-activated transcription factors exerting several functions in development and metabolism. PPARalpha, activated by polyunsaturated fatty acids and fibrates, is implicated in regulation of lipid metabolism, lipoprotein synthesis and metabolism and inflammatory response in liver and other tissues. PPARgamma plays important roles in regulation of proliferation and differentiation of several cell types, including adipose cells. Its activation by thiazolidinediones results in insulin sensibilization and antidiabetic action. Until recently, the physiological functions of PPARdelta remain elusive. The utilization of specific agonists and of appropriate cellular and animal models revealed that PPARdelta has an important role in metabolic adaptation of several tissues to environmental changes. Treatment of obese animals by specific PPARdelta agonists results in normalization of metabolic parameters and reduction of adiposity. The nuclear receptor appeared to be implicated in the regulation of fatty acid burning capacities of skeletal muscle and adipose tissue by controlling the expression of genes involved in fatty acid uptake, beta-oxidation and energy uncoupling. PPARdelta is also implicated in the adaptive metabolic response of skeletal muscle to endurance exercise by controlling the number of oxidative myofibers. Given the results obtained with animal models, PPARdelta agonists may have therapeutic usefulness in metabolic syndrome by increasing fatty acid consumption in skeletal muscle and adipose tissue.

## A novel method for rapid quantification of free and esterified phytosterols in serum using APPI tandem mass spectrometry

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Recent studies suggest that even modest elevations of serum phytosterols may be associated with an increased atherosclerotic risk. However, further large-scale studies are mandatory to prove the role of phytosterols as an additional atherosclerotic risk factor. For this purpose sensitive high throughput analytical methods are necessary.

A novel analytical platform based on liquid chromatography and tandem mass spectrometry (LC-MS/MS) using atmospheric pressure photoionization (APPI) was applied for the simultaneous quantification of free and esterified $\beta$-sitosterol, campesterol, brassicasterol, and stigmasterol.

The total time for sample pretreatment and analysis could be reduced from about three hours (gas chromatography-mass spectrometry, GC-MS) to 15 minutes. The detection limits of the different phytosterols ranged between 0.25 and 0.68 $\mu$g/l. Linear ranges were between 1 and 1000 $\mu$g/l. The within and between-run variability ranged between 1.4 and 9.9%. The analytical sensitivity was at least 150 fold higher compared to GC-MS. Using our novel LC-MS/MS platform, serum phytosterol levels in 49 healthy volunteers were in the same range as those reported by other authors using GC-MS. Campesterol serum mean concentrations of 3.2 mg/l (20) and 5.2 mg/l (1.5–9.7 mg/l) (21) were reported. For $\beta$-sitosterol mean serum concentration s of 2.7 mg/l (20) and 3.6 mg/l (0.8–6.6 mg/l) (21) were found. In comparison, we found a mean total campesterol serum concentration of 4.53 mg/l (1.96–12.27 mg/l) and a mean total $\beta$-sitosterol concentration of 2.43 mg/l (1.01––6.07 mg/l).

In conclusion, our new analytical platform allows a rapid determination of phytosterols and their corresponding esters in small serum volumes without extensive sample pre-treatment. Therefore, our new method is especially suited for large-scale clinical and animal studies to evaluate the role of phytosterols as an additional CHD risk factor.

## Synergistic effects of zeaxanthin and its binding protein in the prevention of lipid membrane oxidation

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There is growing evidence that high levels of the macular xanthophyll carotenoids lutein and zeaxanthin may be protective against visual loss due to age-related macular degeneration, but the actual mechanisms of their protective effects are still poorly understood. We have recently purified, identified and characterized a pi isoform of glutathione S-transferase (GSTP1) as a zeaxanthin-binding protein in the macula of the human eye which specifically and saturably binds to the two forms of zeaxanthin endogenously found in the foveal region (Bhosale et al. 2004). In this report, we studied the synergistic antioxidant role of zeaxanthin and GSTP1 in egg yolk phosphatidylcholine (EYPC) liposomes using hydrophilic 2,2c-azobis (2-methyl-propionamidine) dihydrochloride (AAPH) and lipophilic 2,2c-azobis(2,4-dimethylvaleronitrile) (AMVN) as lipid peroxidant initiators. The two zeaxanthin diastereomers displayed synergistic antioxidant effects against both azo lipid peroxidant initiators when bound to GSTP1. In the presence of GSTP1, non--dietary (3R,3cS-*meso*)-zeaxanthin was observed to be a better antioxidant than dietary (3R,3cR)-zeaxanthin. This effect was found to be independent of the presence of glutathione. Carotenoid degradation profiles indicated that the zeaxanthin diastereomers in association with GSTP1 were more resistant to degradation which may account for the synergistic antioxidant effects.

## Induction of PXR-mediated metabolism by $\beta$-carotene

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Beta-carotene is the major carotenoid in the human nutrition and is present in the human organism in micro-molar concentrations. Besides its function as pro-vitamin A $\beta$-carotene has been shown to be an activator of the human pregnan X receptor (PXR). This PXR is mainly expressed in the liver and an inducer of enzymes involved in phase I, II and III metabolism. The presentation is focused on the evaluation of physiological and nutritional relevance of $\beta$-carotene as an inducer of phase I enzymes in the human organism via PXR-mediated mechanisms. Beneficial and detrimental effects of $\beta$-carotene on xenobiotica metabolism and metabolism of various other derivatives will be discussed.

## Association of adipose and red blood cell lipids with severity of dominant Stargardt macular dystrophy

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The goal of this study was to determine whether or not adipose and red blood cell lipids, particularly long-chain polyunsaturated fatty acids, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are significantly correlated with phenotype in a family with autosomal dominant Stargardt-like macular dystrophy. A mutation in the *ELOVL4* gene is responsible for the macular dystrophy present in this family via its possible involvement in fatty acid elongation in the retina (Bernstein et al. 2001).

The subjects in this study are eighteen adult family members known to have a two base-pair deletion in the *ELOVL4* gene. Each subject received a complete eye examination, including fundus photographs, the results of which were used to grade the subject's severity on a three-tier scale. A blood sample was collected from each subject to examine red blood cell lipids, an indication of short-term dietary fatty acid intake. An adipose tissue sample was collected from fourteen of the eighteen subjects as an indication of long-term dietary fatty acid intake. The adipose and red blood cell lipids were analyzed using capillary gas chromatography with mass spectrometry detection (GC/MS). The subjects also completed a self-administered food frequency questionnaire.

When adipose lipids were analyzed, there was a significant inverse relationship between phenotypic severity and EPA ($p = 0.04$). When red blood cell lipids were analyzed, there were significant inverse relationships between phenotypic severity and EPA ($p = 0.02$) and DHA ($p = 0.04$). There were no significant correlations between dietary fat intake and phenotype when the food frequency questionnaire was analyzed.

These initial results indicate that the phenotypic diversity present in this family may be related to differences in dietary fat intake, as reflected by adipose and red blood cell lipids. To our knowledge, this is the first demonstration that dietary factors can influence the severity of an inherited human macular dystrophy.

Bernstein PS, Tammur J, Singh N et al. (2001) Diverse macular dystrophy phenotype caused by a novel complex mutation in the *ELOVL4* gene. Investigative Ophthalmology and Visual Science 42, 3331–3336.

## The risk polymorphisms of ucp1 and PPARG2 genes in patients with polycystic ovary syndrome

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Aims: The A-3826G gene polymorphism of the uncoupling protein 1 (UCP1) causes the lower expression of UCP1 in the visceral fat and therefore the reduced energy expenditure. Peroxisome proliferator activated receptor gamma 2 (PPARG2) plays a role in the regulation of the adipocyte differentiation and energy balance. The Pro12Ala polymorphism is associated with insulin sensitivity. Since polycystic ovary syndrome (PCOS) is often accompanied with obesity and insulin resistance we decided to study the role of these selected polymorphisms (SNPs) in the pathogenesis of PCOS. Therefore, we determined these polymorphisms in Czech PCOS patients and matched controls. The aim of the study was to compare the allelic and genotypic distribution between these groups and to study the possible association of the polymorphisms with screened biochemical and anthropometric parameters.

Material and methods: The study entered 142 PCOS patients (age 27.0 ± 7.3) and 114 healthy control women (age 27.2 ± 6.1). The well biochemically and anthropometrically characterized PCOS patients and controls underwent the oral glucose tolerance test and the euglycemic hyperinsulinemic clamp in 50 PCOS patients was performed. The A-3826G (AG) and Pro12Ala (PA) SNPs were detected by PCR-RFLP method. The statistical analyses were done using NCSS 2000 software.

Results: The genotype frequencies in PCOS and controls were: UCP1 AA: 53% *vs.* 49.5%; AG: 39% *vs.* 40%, GG: 8% *vs.* 10.5%, PPARG2 PP: 79% *vs.* 76%, PA: 17% *vs.* 23%, AA: 4% *vs.* 1%. Despite the fact that PCOS patients had signif. higher androgene levels, higher BMI, WHR, lipid levels, fasting and stimulated glycaemia and insulinaemia, lower SHBG, 1/HOMA R, Matsuda and QUICKI indeces in comparison to controls, the genotypic frequencies did not differ between the groups (UCP1 $\chi^2 = 0.6$, NS; PPARG2 $\chi^2 = 3.10$, NS). Even though the increase of BMI in G allele carriers in the control group was apparent (UCP1 AA *vs.* AG p = 0.03, AA *vs.* GG p = = 0.02), this trend was not found in the PCOS patients. Pro12Ala polymorphism was not associated with insulin sensitivity.

Conclusion: Our study did not confirm the association of A-3826G and Pro12Ala polymophisms with PCOS.

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## Insulin resistance and the beta-cell function in relation to selected DM 2 candidate genes

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Background and aims: Primary pathogenic causes of diabetes mellitus type 2 (DM2) are still not known, but the final common pathway is the decreased ability of peripheral tissues to respond to insulin (insulin resistance) and at the same time the loss of pancreatic beta-cell ability to compensate it by increase of insulin secretion. Relation between beta-cell function (BF) and insulin resistance (IR), resp. sensitivity (IS), describe disposition indices (DI = BF * IS).

Materials and methods: A total of 292 unrelated subjects with varying degrees of glucose tolerance were clustered on the basis of similarity of disposition index. DI was calculated from stimulated OGTT values of glycemia and insulin (BF: insulinogenic index and IS: Matsuda index, as we described previously in Vrbikova at al. Diabetes Care 25 (7), 2002). The clusters consisting of subjects under 25 percentile (cluster 1) and above 75 percentile (cluster 2) of DI value distribution were compared according to biochemical parameters and anthropometric characteristics. The SNPs of INS VNTR (HphI), FABP2 (HpaI), PPAR gama (HgaI), Beta2AR (MvaI), Beta3AR (MvaI), Kir6.2 (BanII) and UCP1 (BclI) were determined by PCR-RFLP and genotype and allele frequences were compared using non-parametric Mann-Whitney test. Statistical analyses were done by NCSS 2001 program.

Results: Cluster 1 consists of 58 subjects with sex ratio 23 M/35 F, family history of DM2 ratio (first degree) 29 yes/29 no and mean age 37.07 ± 12.55. Cluster 2 consists of 58 subjects with sex ratio 23 M/35 F, family history of DM2 ratio 17 yes/41 no and mean age 32.43 ± 10.87. The differences between clusters were not observed in age and sex ratio, but in cluster 1 were significantly more subjects with positive family history of DM2 than in cluster 2 (p = 0.019). Cluster 1 comprises subjects with significantly higher triacylglycerols (1.24 ± 0.63 *vs.* 0.84 ± 0.41, p = 0.0002) and serum uric acid (292.17 ± 86.47 *vs.* 262.48 ± 74.69, p = 0.047) than subjects in cluster 2. Men and women in cluster 1 had significantly WHR (men 0.89 ± ± 0.08 *vs.* 0.81 ± 0.04 and women 0.77 ± 0.09 *vs.* 0.72 ± 0.05; p = 0.0014, resp. p = 0.015). Women in cluster 1 had also lower muscle to subcutaneous fat ratio than women in cluster 2 (1.51 ± 0.55 *vs.* 1.89 ± 0.68, p = 0.028). Differences in genotype and allele frequecies between clusters were observed only in E23K SNP of Kir6.2 gene (Fisher exact test). In cluster 1 compared to cluster 2 were identified genotype EE in 10

(20.8%) *vs.*18 (37.5%), genotype EK in 27 (56.3%) *vs.* 28 (58.3%) and genotype KK in 11 (22.9%) *vs.* 2 (4.2%) subjects, p = 0.014. Allele frequencies in cluster 1 compared to cluster 2 were E 49% *vs.* E 66.7% and K 51% *vs.* 33.3%, p = 0.013.

Conclusion: Better characteristics of BF and IR in non-diabetic subjects arise from stimulated OGTT values. Clusters generated on the basis of DI value are sifnificantly different in some biochemical and anthropometric parameters. Higher frequency of K allele of Kir6.2 gene in cluster with lower DI is in agreement with described positive association of K allele with decreased BF. Higher frequency of the subjects with positive family history of DM2 in the cluster with lower DI, which provides evidence of the glucose tolerance status, revealed, that stimulated DI is valid criterion for detecting of early stages of impaired glucose tolerance.

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## The KCNJ11 gene polymorphism E23k in Relation To DM2 in Czech population

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Aims: The KCNJ11 (Kir 6.2) gene product is a pore-forming subunit of the inwardly rectifying ATP sensitive K+ channel, which is involved in the regulation of insulin secretion. KCNJ11 E23K polymorphism is considered as a DM2 risk altering SNP. Therefore, we decided to determine allelic and genotypic frequencies of the polymorphism in DM2 patients, in offsprings of DM2 patients, and in healthy adult Czech population. The aim was to compare genotypic distribution between these groups and to study the possible association of the polymorphism with biochemical and anthropometric parameters related to DM2.

Material and methods: The study entered 293 DM2 patients (age 58.67 ± ± 7.10; BMI = 30.55 ± 5.52 kg/m$^2$), 108 offsprings (age 37.91 ± ± 10.54; BMI = 25.35 ± 4.24 kg/m$^2$), and 177 control subjects (age 32.31 ± 10.61; BMI = 23.31 ± 3.67 kg/m$^2$). The E23K substitution was detected by PCR-RFLP method (BanII). The statistical analyses were performed using NCSS 2000 and Statgraphics-plus software.

Results: The 23K allele frequency did not differ between the group of diabetics, offsprings, and controls (38.23%, 40.28%, and 39.55%, resp.). However, genotypic distribution in controls was significantly different in comparison with offsprings (EE/EK/KK: 40.11%/40.68%/19.21% vs. 31.48%/ /56.48%/12.04%, resp. $\chi^2$=7.04; p=0.03). Genotypic frequencies in diabetics (36.52%/50.51%/12.97%) did not differ significantly from controls ($\chi^2$ = 5.46; p = 0.07). The assessment of insulin levels and beta-cell function using the oGTT derived indices in offsprings did not provide any evidence of functional reduction neither in EK nor in KK genotypes. But, in the control group, the KK genotype exhibited in comparison with the EE genotype higher stimulated glucose levels (G30: p = 0.001; G60: p = 0.001; G90: p = 0.003) and lower insulinogenic index (I30-I0/G30-G0: p = 0.012) as well as lower disposition indices.

Conclusions: The association of the KCNJ11 E23K polymorphism with DM2 was not confirmed. Nevertheless, in healthy adult subjects without family history of DM2, the KK carriers exhibited significant reduction in the insulinogenic index as well as in disposition indices.

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## The additive effect of coexistence more than one "susceptibility gene" alleles on development of obesity and insulin resistance

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Several quantitative trait loci have been associated with different features of the metabolic syndrome and the genetic susceptibility have been suggested for multiple polymorphisms in a number candidate of genes. In our study the following gene polymorphism's: PPAR-g2, LPL, FABP-1, b2AR, b3AR, DRD2, MCR-3, MCR-4, UCP-1, HSP-70, TNF-$\alpha$, and apoCIII were analyzed. Several of these genes are involved in direct nutrient interactions, suggesting potential targets for diet modification therapies and the dietary fat is a single most important environmental factor that can modify the impact of the metabolic syndrome. The results of standardized meal (80 g of total fat) on the triglicerides, free fatty acids, insulin and leptin level up to 8 hours were analyzed.

The 250 members of 90 obese families (South Poland) participated in the study. Moreover, because the family members tend to share a similar environment, therefore in our study we can also shown the existence of strong gene-gene interactions which highly influenced the phenotype. For example, the phenotype of the group which characterize existence of two selected homozygous polymorphism's at risk compared to group of the protective genotype demonstrated strong difference in the several components of metabolic syndrome (parameters of obesity, insulin resistance and hypertension). Moreover the additive effect of coexistence more than one "susceptibili-

ty gene" alleles on the development of metabolic syndrome was also observed.

The gene-gene interaction highlight also the importance of studies examining the effects of common genetic polymorphisms within the context of dietary factors. Different genetic backgrounds of various geographic, ethnic or social populations justify the undertaking of large screening programs aimed at the characterization of the genetic variability as it may lead to the development of the targeted nutritional interventions.

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POSTER SESSION

## Prostate cancer and supplementation with fatty acid and beta-carotene: cell cycle regulation

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Prostate cancer is the most common malignancy in men and mortality second only to lung cancer. Prostate tumors are among the most heterogeneous of cancers, both histologically and clinically. The androgen-dependent cancer cells initiate an apoptotic cascade upon androgen ablation, but the androgen-independent cells continue to proliferate. In addition, the different expression of a wild-type p53 and Rb tumor-supressor genes was observed. In prostate cells, the processes of proliferation, differentiation and programmed cell death processes are regulated at least in part by androgens.

Beta carotene is believed to be an effective cancer-preventing nutrient. The ability of beta-carotene to modulate the carcinogenic process, at least *in vitro*, may be due, in part, to its conversion to retinoids in human prostate cell lines and acting through nuclear receptor with a similar mechanism to steroid hormones that have plays an important role in prostate biology. Fatty acids are common components of regular diet also do not remain without effect on a development and progression of prostate cancer. It has been proposed that the omega-6 fatty acids increase the rate of tumor growth. The essential fatty acids, linoleic acid and arachidonic acid (AA), and the AA metabolite $PGE_2$ stimulate tumor growth while oleic acid and the omega-3 fatty acid, eicosapentaenoic acid inhibited growth in cancer cell line. Therefore we studied the effect of BC and AA on prostate cancer.

Material and methods: Studies were carried out on LNCaP androgen-dependent cell line. The cells were treated by 10 $\mu$M b-carotene (BC) in THF/ethanol and by 2.0 $\mu$M arachidonic acids (AA) each separately or added together. The proliferation of cells was determinated by ELISA BrdU and Crystal Violet Test and cytotoxicity using Cytotoxicity Detection Kit (Roche). The expression of genes was studied using microarray analysis (Affymetrix HG-U133A; 22 000 genes), RT-PCR and real-time PCR methods. Protein expression was monitored by Western blot.

Results: The changes in gene expression induced by BC and AA in LNCaP prostate cancer cell line indicated that BC+AA decreased proliferation (~25–35%) of LNCaP prostate cancer cells. The expression of number of genes involved in replication, transcription and translation processes, steroid metabolism (APRIN, FOLH, PTOV, KLK, PLAB, AR, SRD5A, AKR and SREB), cholesterol (PGD) and eicosanoid metabolism was affected. We also observed decreased expression of c-myc, MAD, MAX when BC + AA were used together (decreased proliferation). We noticed the changes in expression of the genes which control G1/S checkpoint — cytokines and CDK (up regulated with BC +AA), Rb and p107 (up regulated with AA + BC) and E2F (down regulated). This observation may explain differences in proliferation of prostate cancer cells treated with BC and/or AA and indicates on strong link between BC/AA treatment and regulation of cell cycle G1/S checkpoint.

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## Different effect of beta-carotene on proliferation of prostate cancer cells

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Beta-carotene is the most important precursor of retinol and other retinoids whereas carotenoids serve as well for several other functions, such as radical quenching, antioxidant and anti-carcinogenic activities and as regulators of the cell proliferation. Retinoids control the growth, differentiation, and apoptosis of cells during embryonic development and throughout life. Acting similarly to hormones, they affect epidermal cell growth, differentiation and sebaceous gland activity, as well as have immunomodulatory and anti-inflammatory properties. The carotenoids present in vegetables and fruits may be responsible for potential cancer-preventing action by inhibiting the growth of tumor cells. Prostate cancer has become the second leading cause of cancer-related death among men in most Western countries. However, relatively little information

concerning native *in vivo* vitamin A dynamics in human prostate tissue is available and even less is known about how certain retinoid therapies and interactions might affect native vitamin A stores or the flux of the vitamin through this tissue. It was shown that high doses of beta-carotene (> 30 $\mu$M) decrease proliferation of prostate cancer cells *in vitro*. However, it is rather doubtful whether such concentration of beta carotene is really accessible at cellular level. We studied the effect of 3, 10 and 20 $\mu$M beta-carotene on proliferation and gene expression in LNCaP and PC-3 prostate cancer cell lines.

Material and methods: Studies were carried out on LNCaP androgen-dependent and PC-3 androgen independent cell lines. The cells were treated by: 3 $\mu$M, 10 $\mu$M and 20 $\mu$M beta--carotene in THF/ethanol 0.3 $\mu$M, 1 $\mu$M, 3 $\mu$M All-Trans Retinoic Acid and 9-cis Retinoic Acid in ethanol. Uptake of BC by cells incubated for 24–72 hrs in various media analysis was studied using HPLC assessment of beta-carotene in media and cell extracts. The proliferation of cells was determined by ELISA BrdU and Crystal Violet Test Cytotoxicity using Cytitoxicity Detection Kit. The expression of genes was studied using microarray analysis (Affymetrix HG-U133A; 22 000 genes), RT--PCR and real-time PCR methods. Expression of RB, CDK2, c-myc, bax, bcl-2 was also studied on protein level (Western blot).

Results: Beta carotene — more efficiently absorbed from medium by androgen sensitive LNCaP cells — increased proliferation of LNCaP cells whereas it had weaker effect on PC-3 cells. Initial global analysis of expression of genes in both cell lines treated with 10 $\mu$M beta-carotene showed remarkable differences in number of responsive genes. Their recognition allows for conclusion that differences between prostate cancer cell lines in response to beta-carotene treatment are due to various androgen sensitivities of LNCaP and PC-3 cells. Detailed analysis of expression of selected genes in beta carotene treated LNCaP cells at the level of mRNA and protein indicated that the observed increase of proliferation could have been the result of slight induction of a few genes affecting proliferation (c-myc, c-jun, c-fos) and apoptosis (bcl-2) with no significant effect on major cell cycle control genes (cdk2, RB, E2F-1).

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## The effect of arachidonic acid and beta-carotene on human melanoma cell growth

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Beta-carotene and other carotenoids in high doses have been shown to have anti-cancer activity, either because of antioxidant activity or due to their possible conversion to retinoic acids. Several studies suggested that essential fatty acid and their metabolites can induce apoptotic death of tumor cell and this action seem to depend on their ability to augment free radical generation and lipid peroxidation. The relation between diet and carcinogenesis has not been presented in sufficient detail to allow recognizing the mechanism of regulation the cell proliferation. In particular no data reported that beta carotene and arachidonic acid were able to control cell proliferation or expression of genes in melanoma cells. Therefore, the effect of physiological concentration of arachidonic acid and beta carotene on cell growth and gene expression in human melanoma cell line A 375 was investigated

Material and methods: Studies were carried on human melanoma cell line A 375 (from tumor metastasis phase). The cells were treated 3 $\mu$M, 10 $\mu$M, 20 $\mu$M beta carotene (BC) and/or by arachidonic acid (AA) — 0.5, 2, and 5 $\mu$M. The proliferation of cells was determined by ELISA BrdU and Crystal Violet Test and cytotoxicity using LDH Test. The gene expression was studied using RT-PCR and Microarray Affymetrix HG-U133a (22 000 genes) and proteins expression (Western Blott).

Results: BC and AA alone, increased incorporation of BrdU but without increase in cell number. BC treatment had no antiproliferative or cytotoxic effect because BC did not affect the genes essential for cellular growth. AA regulated gene expression which are responsible for the G2/M transition in cell cycles (Cdc2 cyclin B1 and Cdc2 – cyclin B2 or Cdc25) but had no antiproliferative effect. Microarray analyses indicated that BC + + AA might affect cell growth by modulation of gene expression that control the transition of cells from G1 to S phase (cyclins and their catalytic subunits and gene involved in replication). This analysis showed no direct correlation with the expression of cdk2 and RB1on protein levels. We also observed decreased incorporation of BrdU without effect on the expression of apoptotic proteins Bax/Bcl2, caspases or inhibi-

tors of apoptosis (surviving). These data suggested, that BC + + AA induced cell cycle delay in G1 phase most likely by changes in physological activity of proteins involved in cell cycle progression.

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## Retinoid signaling promoted by apocarotenals and beta-carotene in BEAS-2B human bronchioepithelial cells

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In two large human intervention trials in smokers, beta--carotene (bc) supplementation was associated with an increased risk for lung cancer. One hypothesis is that apocarotenals are generated via oxidative breakdown of bc by smoke. This would result in inhibited retinoic acid (RA) signaling, leading to squamous metaplasia and cell proliferation. To test this, we compared the effect of retinoids, apocarotenals or bc on RA target gene expression using transcriptomics.

BEAS-2B cells were supplemented with physiological concentrations of all-trans-, 9-cis- and 4-oxo-RA, retinol, 4-oxo-retinol and bc, 4'-, 8'-, 10'- and 12'-apocarotenals for up to 120 h. Cellular contents of the compounds was analysed by HPLC. Gene expression after 72 h of treatment was analyzed with Affymetrix GeneChip® technology. Key findings were verified by TaqMan® real-time RT-PCR.

Bc was taken up in a time- and dose-dependent manner. $\beta$-Apo-10'-carotenal also accumulated time dependently in the cells. No accumulation of retinoids was measured in the cells, except retinol, leading to detectable levels of 9-cisRA and RA. Microarray analysis revealed expected activities of retinoids by upregulating known RA target genes involved in differentiation (TGM2, MucinA3, Keratins), RA signaling (RAR$\alpha$, CRABP2, RARRES1 and RARRES3) and developmental processes (MEIS1, MEOX1 and FOXA1). Bc induced also known RA target genes (TGM2, Mucin3A, Keratins, Cadherin1, RAR$\beta$, MEOX1, FOXA1). There was a high overlap between genes regulated by bc and apocarotenals.

In summary, apocarotenals, as well as bc, increased the expression of numerous RA target genes. Apocarotenals promoted, rather than inhibited retinoid signaling in BEAS-2B cells.

## Beta-carotene beadlets do not cause changes in RARbeta, RARalpha and PCNA expression nor in histology in ferret lungs

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Recently it was reported that high dose oral gavage of crystalline unstabilized beta-carotene in oil to ferrets caused lung metaplasia even in the absence of tobacco smoke exposure. Our goal was to investigate whether stabilized beta-carotene beadlets supplemented via diet would also lead to such adverse effects. Beta-carotene beadlets at 2 mg or 10 mg/kg BW/d were mixed to the ferret chow and given to 4 ferrets per group as a daily bolus over 6 weeks. Controls received placebo beadlets. Beta-carotene and vitamin A metabolites were analyzed in plasma, lung and liver. With quantitative Western blots we estimated the expression levels of the nuclear hormone receptors RARalpha and RARbeta, as well as the proliferating cell nuclear antigen (PCNA) in the lung. Histological evaluation was performed on paraformaldehyde fixed hematoxylin-eosin stained sections and after anti-pancytokeratin immunohistochemistry. We observed a dose dependent increase of beta--carotene concentrations in plasma, lung and liver. All vitamin A metabolites including retinylesters and retinoic acid remained unchanged in plasma, as well as in liver and lung. No apocarotenals were detected in either tissue. The histopathological examination of the lungs did not show any signs of squamous metaplasia or other pathologies. Only spontaneous findings like infiltration of lymphoid cells or alveolitis were observed. The expression level of RARalpha and RARbeta in lung was similar in all 3 groups (control, 2 mg beta-carotene/day and 10 mg/ /beta-carotene/day). The difference of PCNA expression among the 3 groups was not statistical significant.In conclusion, our study did not reveal any molecular or histological changes after 6 weeks of high dose stable beta-carotene beadlet supplementation.

## Effects of oral beta-carotene supplementation on cell cycle markers in the lungs of ferrets

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Epidemiological studies have demonstrated that people that eat more fruits and vegetables (which are rich in carotenoids) and people having higher serum beta-carotene levels have a lower risk of cancer, particularly lung cancer. Beta-carotene may exert a protective role because of its antioxidant activity and/or because of its activity as a precursor of retinoic acid. However, the two main human intervention studies using 20–30 mg daily doses of beta-carotene supplementation revealed an increased risk of lung cancer among smokers and asbestos workers. Previous studies carried out in the ferret, which appears as an appropriate animal model for use in mimicking the conditions of human beta-carotene intervention studies, have reported a mechanistic explanation of the apparent paradoxical effects of beta-carotene on lung cancer, and that its effects could be related to dose: a pharmacological dose of beta-carotene (2.4 mg/kg body weight/day) lead to squamous metaplasia, a pre-cancerous lesion in the lung, and increases the expression of some proto-oncogenes. However, a physiological dose of beta-carotene (0.43 mg/kg body weight/day) did not show these effects, but even provided mild protection again squamous metaplasia in smoke-exposed ferrets. Here, we treated ferrets with two concentrations of oral beta-carotene (0.8 and 3.2 mg/kg body weight/day) for 6 months. Beta-carotene was provided by DSM Nutritional Products as a water soluble formulation (beadlets) containing 0.1% beta-carotene crystalline, 0.015% DL-alpha-tocopherol, 0.04% ascorbyl palmitate, 0.04% corn oil, 0.15% fish gelatine, 0.34% sucrose and 0.315% corn starch. We analysed protein levels of activator protein 1 (c-Jun and c-Fos), c-Myc, cyclin D1, proliferating cellular nuclear antigen (PCNA), and retinoic acid receptor beta (RAR-beta) in lung samples by western blot to test the hypothesis that coordinated effects of beta-carotene on RAR receptors and oncogene expression may explain the effects of beta-carotene. Results show no apparently detrimental effects of beta-carotene on lung cell proliferation and suggest that, in addition to the dose, the formulation (e.g. the presence of alpha-tocopherol or other antioxidant compounds) may be important in determining the pro- or anti-carcinogenic effects of beta-carotene.

## Beta-carotene stability and uptake by human lung bronchial epithelial cells depending on delivery vehicle

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Beta-carotene (BC) has aroused interest about its possible protective or pro-carcinogenic role in lung cancer and cell culture systems are important to evaluate it. Nevertheless, the delivery of BC to cells is difficult due to its hydrophobicity. Different vehicles have been used. Ethanol, liposomes or mixed micelles present problems of insolubility, stability or poor uptake by the cells. Tetrahydrofuran (THF) has been widely used, but it can be toxic and associated with enhancement of tumour formation. Human lipoproteins or BC enriched steer serum are efficient and more physiological vehicles, but not practical. Water dispersible beadlets containing BC have been used as an alternative and produce the greatest accumulation of BC in cells when compared to above vehicles, but other beadlet components (alpha-tocopherol, corn oil, etc) could alter the results. Dimethylsulfoxide (DMSO) could be an alternative since it has low toxicity and it rapidly penetrates across biologic membranes, allowing enhanced penetration of substances accompanying it. We aimed to characterize an appropriate model for delivering all-trans-BC to lung cells in culture. We administered all-trans-BC 5 microM to BEAS-2B cells using beadlets or DMSO, and medium and cell samples were taken at different times. All-trans-BC reached the same levels in the medium (about 3.5 microM) either in beadlets or in DMSO, and, with beadlets, 13-cis-BC was also detected. However the amount of all-trans-BC taken up by the cells was almost the triple when delivered by DMSO compared to beadlets. With both vehicles, intracellular all-trans-BC levels reached its maximum after 24 hours of treatment, and these levels remained the same after 72 hours. The 9-cis and 13-cis forms of BC, as well as other oxidized metabolites, were also detected inside the cells; although their proportion was quite small respect to all-trans-BC, especially when using DMSO. Lastly, a LDH assay showed no toxicity of beadlets, DMSO or BC itself. In conclusion, DMSO seems the most appropriate vehicle for delivering BC to lung cells in vitro, in terms of BC taken up, stability and low formation of derivatives, allowing analysing the effects of BC itself and minimizing interferences from derivatives or other components of the vehicle.

## Comparison of influence of beta-carotene on EPC and HUVEC

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Either endothelial cells covering the inner site of a vessel as well as circulating progenitor cells originating from the bone marrow or other organs may serve as source of endothelial cells for angiogenesis in an adult organism. Beside the growth factors (such as: VEGF, bFGF, TGFbeta, PDGF) or cell/matrix (integrins), cell/cell (VE cadherins, catenins, endoglins, ephrins its receptors and Jagged/Notch pathway) as well the nutrition-related compounds could regulate the most important steps of angiogenesis like proliferation, migration and differentiation. In our experiments we wanted to investigate regulatory influence of compounds from daily diet such as fatty acids and beta-carotene on gene expression, proliferation, migration and differentiation on endothelial progenitor cells (EPC) in comparison to early differentiated endothelial cells (HUVEC).

Material and methods: Primary cultures of human endothelial cells (HUVEC) were isolated from human umbilical vein using collagenase digestion method and were grown in EBM medium with supplement and antibiotics. $AC133^+$ cells were isolated from human cord blood using magnetic microbits (Milteny Biotech) and incubated in EBM medium (with supplement and antibiotics) and with SCF (100 ng/ml) and VEGF (50 ng/ml) six days to obtain endothelial progenitor cells (EPC). Influence of the 24-hour incubation with beta-carotene alone or with arachidonic acid (AA) on the gene expression was measured using oligonucleotide chips (Affymetrix), confirmed by Real--Time PCR and flow-cytometry. Analysis in promotor sequence transcription factor binding sites of regulated genes help us in searching of activated pathways in cells by investigated factors. The cytotoxic effect was checked by estimation of LDH level in tissue medium. Beta-carotene uptake analysis was performed using HPLC. The effect on cell proliferation (rate of DNA synthesis) was measured by BrdU incorporation. Apoptosis was investigated using Apo Fluor TM Green Kit (all caspases activity detection). Chemotaxis was performed using Boyden Chamber System (Becton Dickinson). Angiogenic potency was investigated by the angiogenesis assay in the in vitro and in vivo model.
Results: Beta-carotene was uptaked by EPC and HUVEC was potentiated by arachidonic acid. AA as well BC significantly potentiated basal chemotaxis of EPC and HUVEC cells In spite of microarray-based analysis of gene expression arguing for stimulation of angiogenesis no influence of this compounds on proliferation and apoptosis as well as any influence on the tubule formation in the 3D model *in vitro* were observed. Conclusion. We have demonstrated that BC-in the physiological range of concentrations found in human blood is a potent activator of chemotaxis of early endothelial progenitor cells and HUVEC what is accompanied by the change in the expression of genes mediating cell adhesion and homing, but not activate the final markers of the endothelial differentiation.

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## Proangiogenic activity of beta-carotene is coupled with the activation of endothelial cell chemotaxis

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Endothelial cells play an important role in angiogenesis (formation of new vessels from preexisting ones) which is essential for organogenesis, tissue remodeling but also inflammatory response, carcinogenesis in all periods of our life. Carotenoids present in human tissues function as free radical scavengers, immunomodulators, or some being the substrate for retinol (Vitamin A) and retinoic acid (RA) are regulators of cell fate and differentiation. The study was undertaken to define the direct effects of beta-carotene on endothelial cells in terms of angiogenic activity and regulation of gene expression.

Material and methods: Primary cultures of human endothelial cells (HUVEC) were isolated from human umbilical vein using collagenase digestion method and were grown in EBM medium with supplement and antibiotics. Beta-carotene uptake analysis was performed using HPLC. Influence of the 24-hour incubation with beta-carotene alone or with arachidonic acid (AA) addition on the gene expression was measured using oligonucleotide chips (Affymetrix), and confirmed by Real-Time PCR. Analysis in promotor sequence transcription factor binding sites of regulated genes help us in searching of activated pathways in cells by investigated factors. The effect on cell proliferation (rate of DNA synthesis) was measured by BrdU incorporation. Apoptosis was investigated using Apo Fluor TM Green Kit (all caspases activity detection). Chemotaxis was performed using Boyden Chamber System (Becton Dickinson). Angiogenic potency was investigated by the tubule formation assay in the in vitro 3D matrigel model.

Results: Beta-carotene in the non-toxic concentrations (up to 3 $\mu$M) had no detectable effect on HUVECs proliferation or apoptosis, despite significant influence on the expression patterns of pro- and anti-apoptotic genes. However, beta-carotene did not change the tubulogenic activity of HUVEC in the *in vitro* angiogenesis model, potently accelerated the development of microcapillaries as well as migration of endothelial cells in matrigel plug injected subcutaneously to mice. Potent activation of endothelial cell migration in the *in vitro* model of chemotaxis was observed. The microarray data analysis revealed that the genes involved in cell/cell; cell /matrix adhesion; matrix reorganization; activation of chemotaxis; the G-protein regulated intracellular signaling as well as genes involved in the rapid remodeling of the actin cytoskeleton were the most affected by BC genes in HUVEC.

Conclusion: Beta-carotene in the physiological concentration range stimulates early steps of angiogenic activity of endothelial cells by activation of cellular migration as well as matrix reorganization and decrease of cell adhesion.

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## Influence of squalene treatment on signal transduction

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Squalene, an intermediate metabolite in the synthesis of cholesterol, is established now as natural immunostimulating drug with wound healing activity and radiation protective action. It is known that survival of squalene-fed animals subjected to whole body irradiation in lethal doses was significantly prolonged compared with control-fed ones. This effect of squalene treatment may be explained by confirmation its immunopotentiating radioprotective influence upon molecular mechanisms regulating lymphoid cells functioning.To estimate the effect of squalene administration on signal transduction under conditions of different pathological states, the levels of protein tyrosine phosphatase activities in membrane fractions and cytosol of rat spleen and thymus lymphocytes under conditions of aspirin induced stomach ulcer development and whole body irradiation in different doses were investigated. The enzymes of dephosphorylation play myriad roles in regulation of signal transduction in lymphoid cells. In addition, since the neoplastic transformation of cells and apoptosis development is often accompanied by changes in the state of phosphorylation of tyrosine residues in proteins, the catalytic function of protein tyrosine phosphatases may be a crucial factor in cell differentiation, growth and function regulation. It was shown that the squalene administration caused increasing and normalizing of dephosphorylating abilities of protein tyrosine phosphatases, which were failed in rats with utilizing ulcer.these enzymes. The extension of administration term increased these effects. It was established diverse dose-dependent effects of whole body irradiation on levels of protein tyrosine phosphatase activities. The maximal values of fermentative activities were observed in dose of 0.5 Gy in the most of examined origins. In agreement with the proposes mechanism it was demonstrated that supplementation of squalene to rats has resulted in some shift of maximal values of protein tyrosine phosphatase activities towards higher doses of irradiation in spleen lymphocytes but not in thymus ones. Taken together, the data favor the hypothesis that the squalene treatment potentially increases lymphatic system resistance to damaging action of development such pathological states as stomach ulcer and whole body irradiation.

## Influence of PPAR$\gamma$ agonists on endothelial cells differentiation and on bFGF and VEGF-dependent tubulogenesis

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Background and objective: The Jagged-Notch system plays a crucial role in endothelial cell differentiation and angiogenesis. High *Notch4* expression characterizes proliferating endothelial cells, and Jagged/Notch interaction may regulate the vessel wall cell differentiation. VEGF, a potent stimulator of proliferation, induces the expression of *Jagged 1* in endothelial cells whereas bFGF, a known stimulator of differentiation, down-regulates expression of Notch and Jagged.

The fatty acid and its derivatives activated peroxisome proliferation-activated receptors (PPARs) are important regulators of cellular growth and maturation, including endothelial cell differentiation and angiogenesis. In previous work we demonstrated that administration of the PPAR$\gamma$ agonist: ciglitazone down-regulates expression of *Jagged1* and *Notch4* in endothelial cells and inhibits tubulogenesis and branching of tubules in the 3D matrigel model of angiogenesis. Present work was aimed at elucidating the signaling pathways involved in the PPAR$\gamma$--dependent inhibition of HUVECs proliferation, differentiation and in regulation of Jagged1/Notch4 expression in endothelial cells in response to treatment with VEGF, bFGF and PPAR$\gamma$ agonists.

Material and methods: HUVEC (70% of confluence) were stimulated with VEGF, bFGF or PPAR$\gamma$ activators for 24 hours. Total RNA was isolated and used for real-time RT- PCR analyses (DNA Engine Opticon) to determine *Jagged-1* and *Notch-4* gene expression. Studies of cell differentiation were performed using the 3D matrigel. tubulogenesis model system.

The influence of proangiogenic VEGF (10 ng/ml), and bFGF (10 ng/ml) or antiangiogenic ciglitasone (PPAR$\gamma$ agonist at 1 to 10 $\mu$M) factors on Jagged1/ Notch4 protein expression and the phosphorylation-dependent activation of protein kinases involved in intracellular signaling pathways were detected by Western blot analysis. To determine the role of selected intracellular signaling pathways in differentiation, proliferation and Jagged-1/Notch-4 expression the coincubation with the specific inhibitors of p38MAPK (SB202190 at 10 $\mu$M), PI3K (LY 294002 at 10 $\mu$M) and p42/44 MAPK (U0126 10 $\mu$M) were used.

Results and conclusions: Incubation of HUVECs with PPAR$\gamma$ agonist affected both proliferation as well as differentiation of these cells. We notably found that bFGF and ciglitazone (PPAR$\gamma$ agonist), unlike VEGF, down-regulates *Jagged-1* and *Notch-4* expression. This inhibitory effect required p38 MAPK and Akt signaling pathway. Using the specific kinase inhibitors and the 3D tubulogenesis model we were able for the first time demonstrated that p38MAPK plays a key role in antyangiogenic activity of ciglitazone, the PPAR$\gamma$ agonist.

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## The effect of beta-carotene on differentiation, cytotoxicity, apoptosis and proliferative potential on the three human acute leukemia cell lines

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The influence of beta-carotene (BC) and its derivatives on differentiation, proliferation and apoptosis in three human acute leukemia cell lines was studied. We investigated: (i) the cellular uptake of BC, (ii) the cytotoxicity, (iii) the effect on cell cycle progression and/or apoptosis. The dose- and time dependent pattern of cellular BC uptake in all studied cell lines was seen. We did not observe any cytotoxic effect of BC and ATRA in chosen concentrations. There was only limited effect of BC on gene expression. The microarrray analysis of U-937 cell line exposed to BC for 72 hours showed an increased expression of BAX gene. This finding was confirmed by real-time Q-PCR analysis, and supported by a flow cytometry apoptosis tests. We did not observe any influence of studied components on cellular proliferation. The induction of differentiation after incubation with ATRA in HL-60 cells was noted. The induction of cellular apoptosis by BC was seen in all studied cell lines. We demonstrated that BC used in the concentrations achievable in vivo does not affect the proliferation and differentiation process of studied leukemic cell lines, but can influence and enhance the apoptosis by modulating the expression of the regulatory genes.

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## The potential of beta-carotene to influence the cell fate through the induction or inhibition of apoptosis

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Background: Beta-carotene is rich in human diet both from natural agricultural products and as common food additive. However, some intervention trials have shown that it might trigger or promote cancerogenesis, especially lung cancer.

Objective: To assess the effects of beta-carotene on global gene expression in human models of neoplastic and normal actively proliferating cells.

Material and methods: microarray analysis of gene expression patterns in response to beta-carotene in melanoma A375 and myelomonocytic leukemia U937 cell lines compared to cultured human umbilical vein endothelial cells (HUVECs) followed by real-time quantitative PCR and biological tests for apoptosis (caspase detection and TUNEL).

Results: The experiments with the Affymetrix U133A chips suggested the proapoptotic *BAX* as a common beta-carotene responder in all three cell lines studied. It was upregulated in both A375 and U937 cells, and downregulated in HUVECs. The microarray findings were confirmed by quantitative real-

-time PCR analysis, which also demonstrated concordant changes in other pro- (*BAD*, *BAK1*, *BNIP1*, and *BCLXS*) and anti-apoptotic (*BCLXL*, and *BCL2* isoforms 1 and 2) genes. Tests for apoptosis produced results consistent with gene expression patterns observed in U937 cells and HUVECs, though there was no indication of the predicted induction of apoptosis in the melanoma A375 cells.

Conclusions: Beta-carotene influenced the expression of pro- and antiapoptotic genes in actively proliferating cells. Proapoptotic expression patterns seen in neoplastic cells in response to beta-carotene may (U937) or may not (A375) result in the induction of apoptosis.

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## Effect of catechin and epicatechin on pyruvate dehydrogenase kinase activity in primary culture of rats hepatocytes

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Pyruvate Dehydrogenase Complex (PDH) plays crucial role in Randle cycle, which describes interrelationships between carbohydrate and lipid metabolism in mammals. PDH catalyses irreversible reaction of decarboxylation of pyruvate to acetylCoA.

PDH Complex contains three enzymes, E1 (Pyruvate Dehydrogenase), E2 (Dihydrolipoamide Acetylotransferase) and E3 (Dihydrolipoamide Dehydrogenase) and two regulatory enzymes Pyruvate Dehydrogenase Kinase (PDK) and Pyruvate Dehydrogenase Phosphatase (PDP). PDH complex is mainly regulated by covalent modification. A specific PDH kinase (PDK) catalyses phosphorylation of PDH which results in inactivation whereas a specific PDH phosphatase (PDP) catalyses dephosphorylation and reactivation of PDH complex. PDK is a major factor influencing PDH activity.

It was established that PDK activity is modified by various groups of xenobiotics. One of them are catechins, commonly known plant secondary metabolites ubiquitous in human diet. Current interest of biological activities of catechins is due to their antioxidant properties. It is belived that numerous catechins also improve carbohydrate and lipid metabolism in mammals. Very few studies on influence of catechins on key metabolic enzymes activities were performed. Furthermore, the mechanism of the action of these compounds on molecular level is still unclear. The effect of catechins on PDH activity has not been studied yet.

This study investigated the effect of selected catechins: catechine and epicatechine on PDK activity in primary rat's hepatocytes culture. Liver cells were isolated from male rats and incubated with addition of different doses of catechins. Control hepatocytes were cultured under the same conditions without any catechins. After 24 hours of incubation all cultures were harvested and mitochondria were isolated. In mitochondrial protein extracts PDK activity as well as expression of PDK-2 isoenzyme were assayed. PDK activity was assayed spectrophotometrically by the rate of ATP-dependent inactivation of PDH complex was calculated as the first-order rate constant. Expression of PDK-2 protein was assayed by Western blott analysis.

All tested catechines affected PDK activity and expression of enzyme protein. Adverse correlation between PDK activity and concentration of catechins was demonstrated. All tested catechins remarkably decreased PDK activity and PDK-2 protein amount comparing with control group. The degree of reduction of PDK activity strongly depended on the dose of particular catechins. In conclusion catechins affecting PDK activity modify PDH complex activity and in consequence exert an effect on glucose and lipid metabolism.

## Effect of polyphenols and etanol on pyruvate dehydrogenase kinase activity in primary culture of rats hepatocytes

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Pyruvate Dehydrogenase Complex (PDH) catalyses irreversible reaction of decarboxylation of pyruvate to acetylCoA. PDH complex is a regulatory point of Randle cycle and therefore plays a key role of the overall glucose and lipid disposal in mammals.

PDH complex is mainly regulated by covalent modification. A specific PDH kinase (PDK) inactivate PDH complex by phosphorylation of specific serine residues whereas a specific PDH phosphatase (PDP) exerts adverse effect. PDK is a major factor influencing PDH activity.

The activity of PDK is regulated in short term by mitochondrial NADH/$NAD^+$ and acetylCoA/CoA ratios as well as ATP/ /ADP ratio. Besides, the activity of PDK is modified in long term by nutritional factors includind red wine polyphenols and ethanol.

This study investigated the effect of polyphenols such as caffeic acid + catechine and ethanol on PDK activity in primary rat's hepatocytes culture. Liver cells were isolated from male rats. Primary cultures of isolated hepatocytes were incubated with addition of established doses of polyphenols or ethanol or with the mixture of polyphenols and ethanol. Control hepatocytes were cultured under the same conditions without any additives. After 24 hours of incubation all cultures were harvested and mitochondria were isolated. In mitochondrial protein extract PDK activity as well as expression of PDK-2 isoenzyme were assayed. PDK activity was assayed spectrophotometrically by the rate of ATP-dependent inactivation of PDH complex and calculated as the first-order rate constant. Expression of PDK-2 protein was assayed by Western blott analysis.

It was established that polyphenols caused a reduction in PDK activity as well as a reduction in PDK-2 isoenzyme protein amounts comparing with control group. On the contrary, ethanol increased PDK activity and PDK-2 isoenzyme protein amount, opposite to polyphenols.

To sum up red wine polyphenols may regulate activity of carbohydrate as well as lipid metabolism and change the influence of ethanol on PDH complex activity in mammals.

## UCP1 is expressed in both white and brown adipose tissues of ferrets and is down-regulated after six month daily oral beta-carotene supplementation

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UCP1 is expressed in both white and brown adipose tissues of ferrets and is down-regulated after six month daily oral beta-carotene supplementation. It is known that nutrients are able to affect adipogenesis and/or may have pro-thermogenic properties that can be useful to help control body weight or the medical complications of obesity. Beta-carotene, a vitamin A precursor, could be considered among the different factors influencing energy balance, since in recent years, a growing body of evidence shows that vitamin A derivatives (retinoids) are involved in the control of biological aspects, including adiposity and energy expenditure mechanisms. Here we studied the effects of six month daily oral administration of different doses of supplemental beta-carotene (0.8 mg/kg/day or 3.2 mg/kg/day) in ferrets on body weight and the size of body fat depots and on UCP1 levels (Western blot) in white and brown adipose tissue (WAT and BAT, respectively). Animals that received the high dose of beta-carotene gained more weight than control animals and body weigh at the end of the treatment was 14% higher than controls ($p < 0.05$). No effect was seen with the low dose. Food intake was not affected by beta-carotene supplementation. The size of the subcutaneous inguinal depot in animals treated with the high dose of beta-carotene was significantly higher ($p < 0.05$) than that of animals treated with the low dose and slightly higher than that of controls (19% and 16% higher, respectively). Other depots, particularly gonadal and retroperitoneal WAT, were also slightly higher in animals treated with the high dose of beta-carotene compared with controls, while no apparent effect of beta-carotene was found in the size of parametrial and mesenteric depots. UCP1 was significantly produced in the interscapular BAT and also in the inguinal and retroperitonal WAT depots. Beta-carotene treatment reduced UCP1 protein levels in these depots. In both WAT depots, the decrease was already significant with the low dose of beta-carotene; in BAT the effect was significant with the highest dose. In conclusion, six months of daily oral supplementation with beta-carotene in the ferret produced an increase in body weight and in the size of the inguinal fat depot and decreased UCP1 levels in brown and white adipose tissues.

## Modulation of resistin expression by retinoic acid and vitamin A status

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Aim: Adipocytes secrete many bioactive compounds, among them resistin, a protein that appears to inhibit adipocyte differentiation and to act as a systemic insulin resistance factor. As retinoic acid (RA), vitamin A regulates transcription of target genes (mainly through activation of the retinoid receptors, RARs and RXRs) and impacts on different aspects of the biology of adipose tissues. Our aim was to assess the impact of RA and vitamin A status on resistin expression, gaining insight into the mechanistic aspects and into the functional relevance of this regulation, in terms of changes of glucose tolerance.

Material and methods: Resistin expression was measured in 3T3-L1 adipocytes and primary cultures of brown adipocytes treated with retinoids, and in fat depots and serum of mice acutely treated with RA or chronically fed vitamin A-supplemented diets. Blood parameters and glucose tolerance tests were also conducted.

Results: RA reduced resistin mRNA levels in the adipocyte cell models. The effect was time and dose-dependent, was followed by a reduced secretion of resistin and was reproduced by selective agonists of both RARs and RXRs. RA administration to normal mice resulted in reduced resistin mRNA levels in brown and white adipose tissues, reduced circulating resis-

tin levels, reduced body weight and adiposity and improved glucose tolerance. Resistin expression was also down-regulated after dietary vitamin A supplementation in mice.

Conclusions: RA is an inhibitory signal for resistin expression in adipocytes. Our results are compatible with resistin acting as an insulin resistance factor, but improved glucose tolerance may reflect other effects of RA-treatment, particularly its weigh-reducing effect. The possible relationship among vitamin A status, insulin sensitivity and adipokine expression deserves further studies.

## Retinoic acid treatment induces brown fat cell features in murine white adipose tissues

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Aim: Inefficient oxidation of fuel molecules may contribute to reduced adiposity and is a metabolic feature of brown adipose tissue (BAT), thanks to the specific expression of uncoupling protein 1 (UCP1). Retinoic acid (RA), the carboxylic form of vitamin A, signals transcriptional activation of the UCP1 gene in brown adipocytes. The aim of this work was to analyse the effects of RA-treatment on the expression of UCP1, PGC1alpha (a transcriptional coactivator linked to thermogenesis), PPARalpha (a transcription factor that activates the expression of fatty acid oxidation enzymes) and CPT1 (a target gene of PPARalpha) in murine WAT depots.

Material and methods: The mRNA levels of transcripts of interest were analysed by RT-PCR in adipose tissue depots of adult NMRI male mice treated with vehicle (olive oil) or all-trans RA (at 10, 50 or 100 mg/kg/day, during the 4 days preceding sacrifice). Body weight, fat pad weight and serum non-esterified fatty acids (NEFA) were also determined.

Results: Treatment with RA reduced the weight of all fat pads without a concomitant increase of circulating NEFA levels. In retroperitoneal WAT, RA treatment stimulated UCP1 mRNA, UCP1 protein, PGC1alpha mRNA and PPARalpha mRNA expression. A trend to increased expression of UCP1, CPT1 and PPARalpha mRNA levels was also apparent in inguinal WAT of RA-treated animals.

Conclusion: Our results underscore the idea of adipose tissue plasticity, and suggest that RA may favour to some extent the acquisition of BAT-like properties in WAT depots, promoting the mobilization of WAT fat reserves and the in situ oxidation of the fatty acids released, coupled to the generation of heat.

## Effect of PPAR gamma agonist and high fat diet on the content and composition of ceramides and sphingomielines in the skeletal muscle of the rat

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Effect of PPAR gamma agonist and high fat diet on the content and composition of ceramides and sphingomielines in the skeletal muscle of the rat

The sphingomyelin signaling pathway is an ubiquitous, evolutionarily conserved signalling system. Ceramide which serves as the second messenger in this pathway, is generated from sphingomyelin or by de novo synthesis. However, there are almost no data on metabolism of the ceramide in the skeletal muscle. The aim of the study was to examine the effects of PPAR gamma ligand-pioglitazone on the content and composition of ceramides and sphingomyelins in the skeletal muscles. The experiments were carried out on two groups of male Wistar rats, 200–220 grams of body weight. One group was fed with a standard diet for rodents, and another group was fed with high fat diet ( 48% of fat) for 3 weeks. Each group was divided into two subgroups: 1 — control, 2 — treated with pioglitazone (agonist of PPAR gamma). Pioglitazone were administered by oral gavage with a vehicle in a dose of 3 mg/kg for 14 days. Samples of the soleus, red and white gastrocnemius were taken, frozen in liquid nitrogen and pulverized. Lipids were then extracted with chloroform/methanol (2:1 v/v). Sphingomyelin (SM) and ceramide (CER) were then isolated by means of thin layer chromatography. Lipid bands containing SM and CER were scraped off the plates, methylated and particular fatty acids content were analyzed using gas-liquid chromatography. Eleven different ceramides and sphingomyelins were identified and quantified. They contained the following fatty acid residues: myristic, palmitic, palmitoleic, stearic, oleic, linoleic, arachidonic, nervonic, arachidic, docosapentaenoic and behenic. Total level of analyzed lipids was calculated as sum of individual fatty acids. It has been found that feeding with high fat diet increased total level of SM and CER markedly in all examined muscles. Piogltazone reduced total content of SM and CER in all examined muscles. Both high fat and pioglitazone altered composition of SM and CER and the ratio of saturated to unsaturated fatty acids. It is concluded that activity of the sphingomyelin pathway in the skeletal muscles is under PPAR-gamma control.

## The adiponectin level in patients with familial obesity

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Low adiponectin level is associated with insulin resistance and obesity. Decrease in the synthesis and/or secretion of adiponectin from the adipose tissue is suggested to play a role in the development of atherosclerosis.

The aim of our study was to estimate the link between plasma adiponectin level and postprandial lipemia/glicaemia in patients with familial obesity.

Material and methods: eighty patients from obese families were examined. The measurement of BMI, WHR and the percent of fat tissue was performed (Maltron BF-905). Serum adiponectin, TG, FFA, glucose, insulin, leptin and vWF were determined during oral lipid tolerance test (OLTT) and oral glucose tolerance test (OGTT). Polymorphisms of the obesity candidate-genes': PPARgamma$_2$, beta$_2$AR, beta$_3$ AR, LPL-H, $D_2R$ were also considered as the metabolic determinants.

Results: In the group of 80 patients (43 women, 37 men) the mean BMI was 33.4 kg/m$^2$ (SD ± 7.3), percentage of body fat was 35.2 (SD ± 18.8). The mean adiponectin level in all subjects was 8.28 mg/ml (SD ± 4.9). The level of plasma adiponectin negatively correlated with WHR ($p < 0.05$) and the waist circumference ($p < 0.05$). Plasma adiponectin level was significantly higher in women than in men and in lean subjects compared to obese ones. Moreover men with lower adiponectin demonstrated elevated von Willebrand levels. Significant negative correlation between TG concentration and positive correlation between FFA concentration and plasma adiponectin level was noticed during OLTT. Significantly diminished adiponectin level was observed in patients with increased insulin and glucose concentration during OGTT. HOMA-IR was increased in patients with low adiponectin concentration both in women and men. No correlation was observed between adiponectin plasma level and genes' polymorphisms.

Conclusions: Glycemia/lipemia tolerance parameters are impaired in obese patients with decreased adiponectin level. Impaired postprandial FFA tolerance might be regarded as risk factor of insulin resistance and endothelial dysfunction.

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## The quest for metabolic syndrome in the nutrigenetics era: beta adrenergic gene polymorphisms and weight lowering therapies

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Nutrigenetics and nutrigenomics are promising multidisciplinary fields that focus on studying the interactions between nutritional factors, genetic factors and health outcomes. Their goal is to achieve more efficient individual dietary intervention strategies aimed at preventing disease, improving quality of life and achieving healthy aging. Many studies, using population-based and intervention studies have found evidence for interactions between dietary factors, genetic variants and biochemical markers of metabolic syndrome. Now, the characterization of individuals who may respond better to one type of dietary recommendation than another begins.

The aim of this study was to investigate whether the Gln27Glu polymorphism of the beta2 adrenoreceptor (BAR2) gene contributes to differences in efficacy of weight lowering therapies.

Material and methods: A total of 50 women (age: 32.08 ± ± 6.81) recruited from families with familiar trait of obesity participated in the three month intervention study using diet or diet and exercise programs. BAR2 genotype was determined by polymerase chain reaction followed by ITA I digestion. Anthropometric measurements (weight, height, body fat) and biochemical measurements (serum level of cholesterol, triglyceride, glucose, insulin, leptin) during OLTT and OGTT were performed at the beginning and after therapies.

Results: The frequency of the Glu27 allele did not significantly differ in the obese patients (58%) and lean controls (68%). At the baseline of BMI, body weight, body fat or blood lipid and glucose profiles, there was no significant difference in obese with/without the Glu27 allele of the BAR2. The intervention with diet yielded a triglyceryde reduction and induced a significant difference in BMI (–6.31; –3.17), total cholesterol (–8.89; +1.75), HOMA (–17.34; +0.04) for women without the Glu27 allele and with it, respectively. After diet and physical training therapy significant lower values were found in BMI, body fat % and leptin concentrations among obese carriers of the Glu27 allele. Women with the Glu27 allele showed significant decrease of BMI (–8.3) and fat content (–17.83) after therapy wit diet and physical training.

Thus, anthropometric and biochemical effects of weight-lowering strategies are modulated by a BAR2 polymorphism. Variability at the BAR2 gene is also associated with phenotype of metabolic syndrome. This knowledge should lead to suc-

cessful dietary recommendations partly based on genetic factors that may help to reduce metabolic syndrome risk more efficiently than the current universal recommendations.

This work was supported by Ministry of Informatics and Science grants 4P05D08424; 4P05D01016; 501/Pkl/113/L, 501//Kl/563/L, WŁ130/P/L and NuGo (FP6-2002-FOOD 506360 NuGo" European Nutrigenomics Organisation — Linking genomics, nutrition and health research).

## SNPs as determinants of inter-individual variation in beta-carotene/vitamin A metabolism

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Beta-carotene is a major source of vitamin A in the diet. Several important genes are involved in the metabolism and absorption of beta-carotene. Beta-carotene 15, 15'; monoxygenase is the key enzyme involved in beta-carotene conversion to vitamin A. Beta-carotene absorption and conversion to vitamin A has been found to be highly variable between well nourished healthy individuals. Within the Western population 50% have been classified as low responders to beta-carotene (Hickenbottom et al. 2002). Using SNP detection, genotyping and beta-carotene supplementation studies the aim is to determine if the low responder trait is a result of genetic polymorphisms in key carotenoid cleavage enzymes. Potential SNPs in beta-carotene 15, 15'; monoxygenase were detected by dHPLC using PCR fragments derived from all 11 exons. Several SNPs were identified in beta-carotene 15, 15'; monoxygenase though only SNPs result in amino acid changes confirmed by direct sequencing. Although a correlation between these SNPs and the low responder trait has yet to be made the frequency of both SNPs are over 40% which corresponds with the high percentage of low responders. The second phase of the work will involve a beta-carotene supplementation trail in healthy human volunteers that will correlate response to SNPs identified in beta-carotene 15, 15'; monoxygenase. This will involve volunteers taking a single oral dose of beta-carotene with a fat rich meal and the response measured 3 hours after the meal.

## Switching between physical and chemical mechanism of ROS quenching by carotenoids

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Carotenoids are important natural antioxidants and photoprotective compounds in plant and animal cells. In photosynthesis, apart from light-harvesting function they act as physical quenchers of (bacterio)chlorophyll excited triplet states and of reactive oxygen species, such as singlet oxygen. They might be also involved in chemical quenching when quenching processes proceed further to cause chemical reactions with reactive oxygen, yielding oxygenated derivatives of carotenoids. We have recently shown that the physical quenching of ROS by carotenoids can be replaced by chemical one due to a simple change of solvent. In tetrapyrrole — photosensitised reactions in methanol, all-trans beta-carotene (Car) acts only as the physical quencher of singlet oxygen. A change of the solvent into acetone, completely reroutes the reactions taking place in the system and Car undergoes rapid oxygenation. Seven products of this oxygenation have been identified by RP--HPLC. They accumulate up to six oxygen atoms, while retaining the C40 skeleton. The structural assignments, by MS and 2D-NMR show appearance of stable, cyclic mono- and di-endoperoxides of Car as products of this chemical quenching of singlet oxygen by Car. The resultant endoperoxides are able to promote both the autooxidation and perhaps the oxidation of other species, as the (auto)oxygenation of Car continues in the dark after initial irradiation. Such reactions could be of a great importance in living tissues, where harmful lipid peroxidation may take place in spite of, or under certain conditions, even due to the presence of carotenoids.

## Generation and phenotyping of the beta-carotene 15,15';-monooxygenase KO mouse

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Beta-carotene 15,15;-monooxygenase (BCMO1) is the main enzyme converting the provitamin A carotenoids to retinal. By homologous recombination one allel was mutated in the ES cells. The targeted mutation was then established in the germline and gave rise to the new mouse strain: B6;129S6--Bcmo1tm1DNP. Homozygous offspring was viable and development and growth were comparable with wild type control mice. To assess a possible beta-carotene (bc) effect independently from its provitamin A activity we investigated the effect of bc supplementation and depletion — over more than 100 days — in vitamin A-depleted BCMO1 knockout (KO) and control mice. Mouse body weight was similar between KO and control animals. HPLC analysis of liver and plasma for retinol and bc in KO mice fed a high dose of bc showed tremendous bc accumulation (35 times) compared to their control littermates. Interestingly we also detected little retinol in liver as well as in plasma from KO mice maintained on vitamin A deficient diet and supplemented with bc. More surprisingly we observed a strong homogeneous orange colour of the whole body of the KO mice fed this high bc diet (especially skin, adipose tissue and intestine). This phenotype clearly proves that no or only a truncated form of BCMO1 is expressed in these mice. The small amounts of retinol detected in liver and plasma may be explained by excentric cleavage of bc. Lack of BCMO1 might influence the expression of other genes involved in bc metabolism.

## Lutein, zeaxanthin and lipids as risk and protection factors of age-related macular degeneration

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Objective: Age-related macular degeneration (AMD) is the leading cause of blindness among people older than 65 years of age. Lutein (LUT) and zeaxanthin (ZEA) are the only carotenoids found in macular pigment. They protect the retina from photochemical damage. The main purpose of the study was to assess associations between concentrations of plasma lutein, zeaxanthin and serum lipid fractions and different types of AMD. We also planned to investigate if serum lipids correlate with plasma concentrations of lutein and zeaxanthin.

Material and methods: We examined 215 persons 45 to 94 years of age. Fundus fluorescein angiography and macular photographs were used to divide participants into non-neovascular case group (N-NV) (n = 92) and neovascular case group (NV) (n = 55). Controls (group C) (n = 68) for AMD cases were age- and gender-adjusted persons without any retinal pathology.Plasma carotenoid levels were determined by HPLC and expressed as microgram/L. Serum lipid (total, LDL, HDL cholesterol, triglycerides) concentrations were measured enzymatically and expressed as mg/dL. Kruskal-Wallis ANOVA, U Mann-Whitney test and Spearman rank correlation coefficient were used in statistical analysis.

Results: A significantly lower mean ZEA levels were found in group N-NV compared to controls (16 *vs.* 22.2; $p < 0.004$). Group NV had significally lower LUT (92 *vs.* 127.6; $p < 0.00001$) as well as ZEA (13.2 *vs.* 22.2; $p < 0.004$) concentration in comparison to C group. We found no statistically significant differences in lipid levels between N-NV and C group. Concentrations of total (202.9 *vs.* 221.5; $p < 0.0067$) and LDL cholesterol (126.4 *vs.* 147.7; $p < 0.001$) were significally lower, and triglicerydes (153.2 *vs.* 136.9; $p < 0.02$) significally higher in the group NV compared to controls. We also noted positive correlations between carotenoids (LUT, ZEA) and total ($Rs = +0.27$ and $+0.27$ respectively, $p < 0.0001$), LDL ($Rs = +0.29$ and $+0.3$, $p < 0.0001$), and HDL cholesterol ($Rs = +0.23$, $p < 0.001$ and $+0.15$, $p < 0.03$). Triglycerides were negatively related to LUT and ZEA concentrations ($Rs = -0.19$ and $-0.19$, $p < 0.01$).

Conclusions: The findings suggest that higher plasma xanthophyll concentrations are associated with decreased risk of AMD. The data reveal that hypercholesterolemia is not a risk factor of AMD. Only high concentrations of triglycerides may be related to increased risk of AMD. Positive correlations between carotenoids concentrations and rich in cholesterol lipid fractions (LDL, HDL) may reflect the fact, that xanthophylls are transported by the lipoproteins. The known cardiovascular risk factor may be protection factor of AMD, because of its role in carotenoid metabolism.

## Susceptibility of lipids from different flax cultivars to peroxidation and its lowering by added antioxidants

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Consumption of flax (*Linum usitatissimum*) seeds is beneficial for human health. Flax seeds containing about 40% of oil are the richest, among crop plants, source of polyunsaturated fatty acids (PUFA) essential in human diet. PUFA are highly susceptible to oxidation, thus only certain cultivars (eg. Linola) with low linolenic acid content are suitable for commercial preparation of edible oil, which has, nevertheless, a very short shelf life. To study the factors influencing flax oil stability, oil has been extracted from nine flax cultivars and analyzed. Linola contained about 3% of linolenic acid, while in other analyzed cultivars its content ranged from 52 to 73%. Instead, Linola is rich in linoleic acid (about 75%) which in others cultivars varied from 12 to 18%. The susceptibility to oxidation of extracted oil has been analyzed using two methods (conjugated dienes and malondialdehyde formation). Even the low linolenic acid content Linola oil was easily oxidized. The most resistant to peroxidation was oil extracted from Abby. The potential to reduce peroxidation has been tested using antioxidants (beta-carotene and quercetine) at the concentrations ranging from 10 to 250 $\mu$M. The formation of thiobarbituric acid reactive substances (TBARS) was most efficiently reduced by 25 $\mu$M of both beta-carotene and quercetine. Higher concentrations of beta-carotene increased level of TBARS. The efficiency of beta-carotene and quercetine varied depending on analyzed cultivar probably due to intrinsic content of antioxidants.

## Differences in all-trans beta--carotene (BC) uptake and eccentric cleavage by human endothelial and neoplastic cell lines

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*In vitro* culture of various cell lines serves as an important model for investigation of the biological activity of beta-carotene (BC). This effect depends on cellular uptake and metabolism of beta-carotene. This study was aimed to investigate the ability of the different human cell lines to BC uptake and metabolism by the eccentric cleavage and oxidation. The human normal cell line HUVEC (human umbilical vein endothelial cells) and human neoplastic cell lines (leukemia, prostate and melanoma) were exposed to all-trans beta-carotene. 3 $\mu$M beta-carotene was supplemented alone or in combination with 3 $\mu$M arachidonic acid (AA) to HUVEC, HL60, WM35, A375, PC-3 and LNCaP cell lines. In the second medium, 10 $\mu$M beta--carotene was supplemented alone or in combination with 3 $\mu$M AA to HL60, TF-1, U937, WM35, A375, PC-3 and LNCaP cell lines. The incubation time was 24 hours, 48 hours and 72 hours. Both medium as well as cell lysates after extraction were analysed by HPLC for the BC and its metabolite content. Cellular BC content in response to supplementation with beta--carotene alone (AUC BC) or in combination with arachidonic acid (AUC BC/AA) at three time intervals was calculated as the area under the curve (AUC). Eccentric cleavage and oxidation products formed from beta-carotene i.e. beta-carotene-monoepoxide content (Epoxide ratio), and sum of 12'-apocarotenal, 8'-apocarotenal and 4'-$\beta$-apocarotenal (Apo ratio), were expressed as the percent of the total cellular all-trans beta-carotene. Significant differences in BC accumulation between the various cell types. led to following categorization: L — low BC uptake, M — medium and H — high beta-carotene uptake according to AUC value of the cellular BC content: L: $< 25^{th}$ percentile, M: $< 25^{th}$ AUC $< 50^{th}$ percentile and H: $> 50^{th}$ percentile respectively. In several cell lines AA augmented the cellular BC uptake. HUVEC and human promyelocytic leukemia HL-60 cell line were classified to L group. Human myelomonocytic U937 cells and human erythroleukemic TF-1 cell line were classified to medium-uptake group. The melanoma cell lines: WM 35 from a primary radial growth

phase lesion, and A375 from metastatic sites as well as the prostate cancer cell lines: LNCaP and the androgen-sensitive PC-3, represented high-uptake group for both beta-carotene concentrations. Combined supplementation i.e. arachidonic acid with beta-carotene, reversed the uptake relation between both prostate cell lines and melanoma lines. A significant inverse correlation between beta-carotene uptake (AUC BC) and Epoxy ratio and Apo ratio was observed in all cells, at both BC concentrations used. In summary, we found that BC dissolved in freshly purified THF in "pharmacological" , non-toxic concentrations is differentially absorbed by cell lines. Cells with low BC uptake (endothelial and leukemia cells) are characterized by a higher BC catabolism rate in comparison to cells with high BC uptake such as prostate carcinoma and melanoma.

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## Effects of dietary lycopene on lipid parameters and yolk coloration in Japanese quail

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Lycopene is a member of the carotenoid family as an acyclic isomer of beta-carotene but has no vitamin A activity. This pigment is responsible for the red color of tomato and its products. Beside the coloration effects lycopene has one of the highest antioxidant activity of all the carotenoids but non-antioxidant mechanisms have also been proposed such as upregulation of gap junction protein expressions, suppression of phosphorylation, inhibition of cholesterol synthesis and cell division. Because lycopene may have some health benefits a lot of studies focused its effects. Most of the studies deal with the aspects of human health (i.e. prostate and lung cancer) and less in animal science. Some effects of lycopene were investigated in model experiments on Japanese quails by our group. The effects of yolk coloration, some parameters of lipid metabolism and antioxidant status and non challenged avian immunoglobulin (IgY) titers were investigated of quails kept on fodder containing natural originated lycopene supplement in the form of tomato concentrate for 4 weeks. According the results lycopene has a valuable pigmentation effect of egg yolk. Plasma and liver cholesterol decreased in lycopene supplemented animals. The antioxidative status was evaluated using thiobarbituric acid--reactive substance (TBARS) and ferro-reducing ability of plasma (FRAP) methods. These parameters and the IgY titers were not changed markedly. We can conclude that the lycopene containing tomato produce, maybe some by-product of processing could be use as beneficial factor in the poultry nutrition.

## Beta-carotene and cigarette smoke: a chemical and biological approach

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Human intervention trials have shown that supplemental beta-carotene increases cancer risk in smokers but the mechanism underlying this effect is still unknown. The chemical and biological effect of cigarette smoke and beta-carotene are poorly understood. Only a relatively small number of studies have attempted to determine the degradation pathways involved when beta-carotene is challenged with cigarette smoke (and in particular identifying the products of such reactions). The aims of this study were to establish the oxidation products formed when beta-carotene was exposed to cigarette smoke and to investigate the effect on cell growth and apoptosis on two human lung cell lines a) when cells are exposed to beta--carotene and cigarette smoke and b) when beta-carotene has previously been oxidised by cigarette smoke. Beta-carotene was exposed to filtered cigarette smoke and its oxidation products were analysed using a HPLC employing a C30 reversed-phase YMC column with diode array detection. The chemical structure of these components was determined by LC-MS and by GC-MS analysis. Two different human lung cell lines (CORL--24 and NCIH-727) were exposed to cigarette smoke in combination with beta-carotene and also to beta-carotene that has been previously oxidised by cigarette smoke at concentrations of 1, 5 and 10 $\mu$M respectively. Oxidation using whole cigarette smoke resulted in considerable bleaching of beta-carotene coupled with the presence of a range of oxidation products being detected including 4-nitro-beta-carotene, cis-isomers and low molecular volatile oxidation products such as beta-cyclocitral, beta-ionone and 5, 6 epoxy-beta-ionone. The first exposure of transformed cells to beta-carotene and cigarette smoke resulted in an inhibition of growth particularly when cells were previously supplemented with higher doses of beta-carotene. When only 1 $\mu$M beta-carotene was used it exhibited less inhibition of growth compared to the cells only exposed to smoke. It seems to suggest that the combination of cigarette smoke and beta-carotene has a major effect in promoting the apoptosis of these cells with the lower dose of beta-carotene being more protective against apoptosis, when exposed to cigarette smoke.

## Chemical composition of dried biomass of *Blakeslea trispora*

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The problem of obtaining of natural carotenoids from relatively cheap sources of raw materials is rather important now. One of super-producers of beta-carotene is a mucor fungus *Blakeslea trispora*, processed commercially by microbiological synthesis. Besides beta-carotene, products of processing of the fungus are rich in other vitamins, polyunsaturated fatty acids, minerals, micro- and macro-elements. The objective of the present research was the definition of chemical composition of products of processing of the fungus Blakeslea trispora. The materials for the investigations were the products, commercially produced by the research and production enterprise „VITAN" (Dnepropetrovsk region, Ukraine): the dried biomass and products of its extraction, i.e. the beta-carotene oil solution (0.2% of pure beta-carotene) and beta-carotene suspension in fat (30% of pure beta-carotene). The analyses were carried out in the Institute of Biochemistry (Kiev, Ukraine). It was determined, that the biomass contains from 4 up to 9% of beta-carotene. Besides beta-carotene, the biomass contains vitamins B1 (0.1–0.2 mg%), B2 (0.2–0.9 mg%), B6 (0.7–0.9 mg%), B9 (0.35–0.45 mg%), C (20–25 mg%), PP (1–1.5 mg%), E (15–28 mg%). The phenotypic correlation between the percentage of beta-carotene in the biomass and some vitamins has been investigated: B1 ($r = -0.62$), B2 ($r = 0.98$), B6 ($r = 0.98$), B9 ($r = 0.38$), C ($r = 0.41$), E ($r = 0.24$). The biomass also contains the following esential fatty acids: oleinic (~30%), linolic (~50%), linolenic (~0,5%), arachidonic (~0,2%); and free amino acids: glutamic (~12 mg%), lysine (~10 mg%), leucine (~8 mg%), valine (~6 mg%), isoleucine (~5 mg%), glutamine (~5 mg%), phenyl alanine (~5 mg%). The investigation of the oil solutions showed the high correlation ($r = 0.98$) between the beta-carotene percentage and vitamin E. Besides vitamin E, the oil solutions contain B6 (0.62 mg%), B9 (0.16 mg%), PP (1 mg%). In fatty acid composition the linolic (~60%) and oleinic (~30%) acids prevailed. Besides these, the palmitic (~7%), arachidonic (~1%) and linolenic (~0,5%) acids have been defined.Thus, it is possible to conclude, that the products of processing of the fungus *Blakeslea trispora* besides the ability to produce beta-carotene, are depositors of a unique polyvitaminic complex that makes these valuable edible and fodder preparations.

## Profiles of lipids in fruit-bodies of some edible mushrooms

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The lipids important in nutrition are of three classes: triglycerides (called fats), phospholipids, and sterols. The predominant lipids in foods are triglycerides, and sterols and their esters. Fatty acids vary in the length of their carbon chains, their degree of unsaturation, and location of their double bond(s). Mushrooms as food are a valuable source of vitamins (mainly of B group and C), micro elements, and protein (content of proteins is low, but of high quality (NPU) and amino acids profile, close to animal proteins). Also content of fiber (chitin, lowering level of animal sterols and triglycerides in digested foods, as well as polysaccharides, substances of medicinal value) is appropriate for diet. Hard-digestibility is, for actual knowledge, rather a myth derived from mycophoby of parts of Europe and North America. So we decide to investigate rather a weekly known problem of lipid profiles of edible mushrooms. In the material presented, we analyzed contents of various types of lipids in fruit-bodies of edible mushrooms: tree wilde specia (*Boletus edulis*, *Cantharellus cibarius*, and *Armilariella mellea* — wood-degrading one) and four cultivated specia (*Agaricus bisporus* — saprotrophic one, and *Lentinus edodes*, *Pleurotus ostreatus* and *Pholiota nameko* — wood-degrading ones). We confirm that content of crude fat in mushrooms is relatively low (2–9%), higher in wood-degrading specia. In fatty acids we demonstrated presence of omega-6 acids (mainly linoleic acid). In complex lipids we obtained high content of cerebrosides, valuable for their biological activity. In sterols main constituents were, according to species, ergosterol (and vitamin D2 derivatives), fungisterol, brassicasterol, lanosterol (and lanostan derivatives), squalen, and triterpene acids. The last ones are especially of great medicinal value and have potential pharmacological activity. Our investigations confirmed the dietary and medicinal value of mushrooms as functional foods and nutraceuticals, also in scope of dietary lipids.

# Index

# Acta Angiologica
# Editorial Policy & Instructions for Authors

## EDITORIAL POLICY AND GENERAL INFORMATION

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**(book reference with different author and editor)** Rosen MR (1992) Principles of cardiac electrophysiology. In: Internal Medicine. Kelley WN ed. J.B. Lippincott Company, Philadelphia, 90–95.

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# Acta Angiologica
# Zasady edycji oraz informacje dla autorów

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Regulamin zgłaszania artykułów do druku opracowano na podstawie "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" N. Engl. J. Med. 1997; 336: 309–315.

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**Prace powinny mieć następującą strukturę:**
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**Przykład**
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*Książki.* W wypadku cytowanych książek należy wymienić: kolejny numer pozycji, nazwiska autorów, rok wydania, tytuł, wydawcę, miejsce wydania. Powołując się na treść rozdziału książki, należy podać: nazwisko autora, inicjały imion, rok wydania, tytuł rozdziału, nazwisko autora (redaktora) książki, inicjały imion, tytuł książki, wydawcę, miejsce wydania, przedział stron.

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