

## **COST ACTION FA0602**

# **MITOFOOD**

Kings College, University of Aberdeen, Scotland, UK

19<sup>th</sup> – 21<sup>st</sup> May 2010



Chairman

**Jaap Keijer**

Local Organisers

**John Speakman**  
**Colin Selman**

# *PROGRAMME*

**Wednesday 19<sup>th</sup> May 2010**

1900-2100h : Meeting room. Maryculter House Hotel, management committee meeting

## **Draft Agenda**

### **7<sup>th</sup> Management Committee Meeting**

#### **COST Action FA0602**

#### **Bioactive food components, mitochondrial function and health**

#### **Aberdeen (United Kingdom), from 20/05/2010 to 21/05/2010**

0. Welcome to participants
1. Adoption of agenda
2. Minutes of last meeting
3. Matters arising
4. Report from the COST Office
  - News from the COST Office
  - Status of Action, including participating countries
  - Budget Status, budget planning and allocation process
5. Progress report of working groups
6. Action planning
  - 6.1 Annual Progress Conference (preparation and/or feedback from DC)
  - 6.2 Action Budget Planning
  - 6.3 Action Planning (including meetings)
    - 6.3.1 Location and date of next meeting
    - 6.3.2 Long-term planning (including anticipated locations and dates of future meetings)
7. STSM status, applications
8. Publications, dissemination and outreach activities
9. Request for new members
10. Promotion of gender balance and of Early Stage Researchers (ESR)
11. Non-COST country participations
12. Web news
13. AOB
14. Closing

**COST ACTION ATTENDEES with contact details**

1. Anna Ardevol [anna.ardevol@urv.cat](mailto:anna.ardevol@urv.cat)
2. Thierry Arnould [thierry.arnould@fundp.ac.be](mailto:thierry.arnould@fundp.ac.be)
3. Ioanna Andreadou [jandread@pharm.uoa.gr](mailto:jandread@pharm.uoa.gr)
4. Torsten Bohn [bohn@lippmann.lu](mailto:bohn@lippmann.lu)
5. Vilma Borutaite [vilbor@vector.kmu.lt](mailto:vilbor@vector.kmu.lt)
6. Biljana Buzadzic [buzadzic@ibiss.bg.ac.rs](mailto:buzadzic@ibiss.bg.ac.rs)
7. Aldona Dembinska-Kiec [mbkiec@cyf-kr.edu.pl](mailto:mbkiec@cyf-kr.edu.pl)
8. Gérard Cabello [cabello@supagro.inra.fr](mailto:cabello@supagro.inra.fr)
9. Barbara Cannon [barbara.cannon@wgi.su.se](mailto:barbara.cannon@wgi.su.se)
10. Jerzy Duszynski [j dus@nencki.gov.pl](mailto:j dus@nencki.gov.pl)
11. Pavel Flachs
12. Nikolas Fokialakis [fokialakis@pharm.uoa.gr](mailto:fokialakis@pharm.uoa.gr)
13. Thomas Gettys [Thomas.Gettys@pbrc.edu](mailto:Thomas.Gettys@pbrc.edu)
14. Erich Gnaiger [erich.gnaiger@oroboros.at](mailto:erich.gnaiger@oroboros.at)
15. Dimitrina Katcheva [dikacheva@hotmail.com](mailto:dikacheva@hotmail.com)
16. Jaap Keiger [Jaap.Keijer@wur.nl](mailto:Jaap.Keijer@wur.nl)
17. Elena Kistanova [kistanova@gmail.com](mailto:kistanova@gmail.com)
18. Susanne Klaus [klaus@dife.de](mailto:klaus@dife.de)
19. Jan Kopecky [kopecky@biomed.cas.cz](mailto:kopecky@biomed.cas.cz)
20. Thierry Letellier [thierry.letellier@u-bordeaux2.fr](mailto:thierry.letellier@u-bordeaux2.fr)
21. John Mathers [john.mathers@newcastle.ac.uk](mailto:john.mathers@newcastle.ac.uk)
22. Danina Muntean [daninamuntean@gmail.com](mailto:daninamuntean@gmail.com)
23. Johanna Mihaly [johanna@dote.hu](mailto:johanna@dote.hu)
24. Jan Nedergaard [jan@metabol.su.se](mailto:jan@metabol.su.se)
25. Carlos Palmeira [palmeira@zoo.uc.pt](mailto:palmeira@zoo.uc.pt)
26. Manuel Portero-Otin [manuel.portero@mex.udl.cat](mailto:manuel.portero@mex.udl.cat)
27. Jolita Radusiene [jolitar@takas.lt](mailto:jolitar@takas.lt)
28. Holly van Remmen [VANREMMEN@uthscsa.edu](mailto:VANREMMEN@uthscsa.edu)
29. Michael Ristow [mristow@mristow.org](mailto:mristow@mristow.org)
30. Christophe Rocher
31. Ralph Ruehl [ralphruehl@web.de](mailto:ralphruehl@web.de)
32. Irinia Shabalina [irina.shabalina@wgi.su.se](mailto:irina.shabalina@wgi.su.se)
33. Ana Stancic [anavasilijevic@ibiss.bg.ac.rs](mailto:anavasilijevic@ibiss.bg.ac.rs)
34. Joanna Szczepanowska [j.szczepanowska@nencki.gov.pl](mailto:j.szczepanowska@nencki.gov.pl)
35. Jozef and Barbara Ukropec [jozef.ukropec@savba.sk](mailto:jozef.ukropec@savba.sk)
36. Uwe Wenzel [Uwe.Wenzel@ernaehrung.uni-giessen.de](mailto:Uwe.Wenzel@ernaehrung.uni-giessen.de)

### University of Aberdeen Attendees

1. Mia Aitchieson [r09ma9@abdn.ac.uk](mailto:r09ma9@abdn.ac.uk)
2. Ruth Banks [r.e.banks@abdn.ac.uk](mailto:r.e.banks@abdn.ac.uk)
3. Mirela Delibegovic [m.delibegovic@abdn.ac.uk](mailto:m.delibegovic@abdn.ac.uk)
4. Janice Drew [j.drew@abdn.ac.uk](mailto:j.drew@abdn.ac.uk)
5. Stuart Gray [s.r.gray@abdn.ac.uk](mailto:s.r.gray@abdn.ac.uk)
6. Aqeel al Jothery [aqeel19782000@gmail.com](mailto:aqeel19782000@gmail.com)
7. Andreas Kolb [a.kolb@abdn.ac.uk](mailto:a.kolb@abdn.ac.uk)
8. Ela Krol [e.krol@abdn.ac.uk](mailto:e.krol@abdn.ac.uk)
9. Damon Lowes [d.lowes@abdn.ac.uk](mailto:d.lowes@abdn.ac.uk)
10. Debbie McLaggan [d.mclaggan@abdn.ac.uk](mailto:d.mclaggan@abdn.ac.uk)
11. Beverly Minter
12. Sharon Mitchell [s.e.mitchell@abdn.ac.uk](mailto:s.e.mitchell@abdn.ac.uk)
13. Mike Rogers [m.j.rogers@abdn.ac.uk](mailto:m.j.rogers@abdn.ac.uk)
14. Vanessa Rungapamestry [v.rungapamestry@abdn.ac.uk](mailto:v.rungapamestry@abdn.ac.uk)
15. Colin Selman [c.selman@abdn.ac.uk](mailto:c.selman@abdn.ac.uk)
16. Rachel Sinclair [r.sinclair@abdn.ac.uk](mailto:r.sinclair@abdn.ac.uk)
17. John Speakman [j.speakman@abdn.ac.uk](mailto:j.speakman@abdn.ac.uk)
18. Cathy Wyse [c.wyse@abdn.ac.uk](mailto:c.wyse@abdn.ac.uk)
19. Lina Zhang [lina.zhang@abdn.ac.uk](mailto:lina.zhang@abdn.ac.uk)



Maryculter House hotel

**PROGRAMME****Thursday 20<sup>th</sup> May 2010**

0700-0830h Breakfast at the hotel.

0830h Pick up by bus at hotel. Transfer to Linklater rooms, Kings college, University of Aberdeen  
Arrival c 0900h depending on traffic

**0920-0930h Housekeeping : John Speakman****Session one : Chairman : Jaap Keijer**

0930 – 0950h **Manuel Portero-Otin [A1]**  
Protein oxidative damage and mitochondrial dysfunction in a model of insulin resistance: modulation by diet

0950- 1020h **Gerard Cabello [A2]**  
Influence of an hypercaloric diet on mitochondrial activity : involvement in obesity and metabolic syndrome.

1020-1040h **Aldona Dembinska-Kiec [A3]**  
Lipid droplets, mitochondria and cell autophagy in adipose stromal vascular fraction cells

1040-1100h MORNING COFFEE : Linklater rooms

**Session two: Chairman: Danina Muntean**

1100-1200h **Plenary lecture**  
**Thomas Gettys [A4]**  
Metabolic Consequences of Tissue-Specific Remodeling of Lipid Metabolism by Dietary Methionine Restriction  
Pennington Biomedical Research Center, Baton Rouge, USA

1200-1220h **Thierry Letillier**  
Mitochondrial Haplogroup and pathologies : application for nutrition

1220 – 1400h LUNCH

**Session three: Chairman: John Speakman**

1400-1420h **Erich Gnaiger[A5]**  
Fast and food - body mass index and mitochondrial capacity in human skeletal muscle

1420-1520h **Plenary lecture**  
**Holly van Remmen**  
Barshop Institute San Antonio, Texas, USA  
The free-radical damage theory of ageing: Dead or on life support?

1520- 1540h AFTERNOON TEA

**Session four: Chairman : Jan Nedergaard**

1540-1600h **Anna Ardevol [A6]**  
 Procyanidins limit insulin secretion by modifying the mitochondrial membrane potential

1600-1620h **Uwe Wenzel [A7]**  
 Polyphenols from edible plants and their potential to inhibit glucose-induced mitochondrial stress in the nematode *Caenorhabditis elegans*

1620-1640h **Josef Ukropec [A8]**  
 Hypoxia signalling in the adipose tissue of individuals with the Chronic Obstructive Pulmonary Disease: Insulin sensitivity and adaptation of adipose tissue to systemic hypoxemia in COPD

1640-1700h **Suzanne Klaus [A9]**  
 Muscle mitochondria uncoupling leads to healthy ageing in mice

1700-1720h **John Mathers [A10]**  
 Mitochondrial mutations in the human colon during ageing

Session ends at c. 1730h

1730h bus pick-up at Linklater rooms transfer to hotel  
 c.1800h arrive at hotel (30 mins to freshen up and get changed)

1830h BUS pick-up at hotel transfer to Dunnottar  
 1900 – 1945h Walk to restaurant



1945h – c2300h CONFERENCE DINNER  
 Tolbooth restaurant, Stonehaven

c. 2300h BUS pick up at restaurant  
c2340h arrival back at hotel

**Friday 21st May 2010**

0700-0830h Breakfast at the hotel.

0830h Pick up by bus at hotel. Transfer to Linklater rooms,  
Kings college, University of Aberdeen

Arrival c 0900h depending on traffic

**0920-0930h Housekeeping: John Speakman**

**Session five: Chairman: Colin Selman**

0920-1020h **Plenary lecture**  
**Michael Ristow**  
University of Jena  
Antioxidants and ageing

1020-1040h **Vilma Borutaite [A11]**  
Different effects of standardized *Ginkgo biloba* extract on heart and liver mitochondria

1040-1100h MORNING COFFEE

**Session six: Chairman: Barbara Cannon**

1100-1120h **Jan Kopecky [A12]**  
Obscure mechanisms for differential adaptation of mice of identical genetic background to high-fat diet

1120-1140h **Thierry Arnould [A13]**  
Qualitative and quantitative analysis of mitochondrial population in 3T3-L1 adipocytes responding to a mild mitochondrial uncoupling: a mitoproteomic approach

1140-1200h **Torsten Bohn [A14]**  
Polyphenols and carotenoids - bioavailability and relation to chronic diseases

1200-1220h **Irina Shabalina [A15]**  
Mitochondrial function and premature ageing: Impaired function of mitochondria in tissues from mice expressing defective mitochondrial DNA polymerase

1220-1240h **Ralph Ruehl**  
Pufa metabolites and inflammation / obesity

1240-1300h Closing remarks  
**John Speakman**

1300-1415h LUNCH

1415h Bus leaves to airport (transfer takes about 20 minutes)

ABSTRACTS DAY ONE [A1 to A10]

[A1]

**Protein oxidative damage and mitochondrial dysfunction in a model of insulin resistance: modulation by diet**

José CE Serrano, Jordi Boada, Hugo Gonzalo, Anna Casañé, Meritxell Martin, Alberto Espinel<sup>1</sup>, Marco Antonio Delgado<sup>1</sup>, Reinald Pamplona and **Manuel Portero-Otin**

From the Nutren Group (PCiTAL-IRBLLEIDA-UdL), Lleida, Spain and <sup>1</sup>Grupo Leche Pascual, Aranda de Duero, Spain

Insulin resistance (IR) has been linked to redox alterations in tissues involved in energy homeostasis, such as liver, adipose tissue and skeletal muscle. Thus, diets enriched in compounds showing antioxidant properties can be proposed as a preventive measurement for IR. However, many compounds with low antioxidant potency in vitro behave as potent redox modulators in vivo, thus being good candidates for IR prevention. To evaluate this hypothesis we tested the effect of a soy drink in IR models, both in vitro and in vivo. CD1 mice were rendered insulin-resistant under an hyperlipidic diet. Interestingly, the inclusion of soy -with a low in vitro antioxidant score- in this diet prevented IR development. Mass-spectra based metabolomic analyses was used to ascertain mechanisms involved in this effect. These results insinuated the involvement of mitochondrial fatty acid metabolism. Western-blot analyses in skeletal muscle demonstrated that soy drink changed the amount of peptides representative of respiratory complexes II and III, together with a paradoxical increase in protein glycoxidative and lipoxidative damage. When looking at AMPK, soy drink was able to partially prevent the diminished concentration of this protein in hyperlipidemic mice. Consequently, we detected increased amounts of porin which suggested increased mitochondrial mass as a part of the soy-induced changes. Functional analyses in skeletal muscle were compatible with a soy-induced adaptation directed at using FADH reducing equivalents in mitochondrial respiratory chain. In this way, AMPK-induced enhancement of beta-oxidation would not be uncoupled to respiration, allowing to diminish potentially IR-linked lipid intermediates in muscle. This pathway can constitute a novel mechanism connecting soy intake and IR prevention.

[A2]

**INFLUENCE OF UMBALANCED DIETS ON MITOCHONDRIAL ACTIVITY: INVOLVEMENT IN OBESITY AND METABOLIC SYNDROME MitHyCal consortium (ANR, French National Agency)**

Endocrinologie mitochondriale et Nutrition, UMR 866 INRA Montpellier; CPID, UMR 5160 CNRS, Montpellier; UMR 5018 Université Paul Sabatier, Toulouse; Unité métabolisme protéino-énergétique, UMR 1019 INRA Clermont-Fd; Laboratoire de Bioénergétique fondamentale et appliquée, INSERM Grenoble; UMR 4104 INSERM/CNRS, Institut Cochin, Paris.

Today, 13 million of french people are overweight and 4.2 millions are obese, due to an increasing frequency of nutritional imbalances. This increasing frequency of obesity is associated to a permanent rise in the number of patients with a metabolic syndrome. The aim of this proposal was to better understand the involvement of mitochondrial activity alterations in the occurrence of a metabolic syndrome with and without obesity. This project addressed the following questions: i) what are the

differential consequences of unbalanced diets inducing an association or a dissociation of obesity and metabolic syndrome on mitochondrial activity and insulin signalling ? ii) what are the consequences of experimental changes in mitochondrial activity on the phenotype induced by diet imbalances ? iii) what role plays ROS production in these diet-induced adaptations ? *In vivo* studies have involved different diets (High fructose -HFr- and High fat/high sucrose -HF/HS- in rats; High Fat -HF- in mice) inducing different phenotypes in rats and mice associating or not obesity and insulin resistance. Mitochondrial activity, ROS production and several metabolic and hormonal blood parameters have been studied. In addition, four groups of patients were constituted on the basis of their abdominal adiposity, and similar aspects were studied. Lastly, genetical changes of mitochondrial activity have been induced in mice (AIF -/-, liver overexpression of a CPT1 insensitive to malonyl CoA, p43 -/-) with the aim to characterize their influence on glucose metabolism and the phenotype obtained when the animals were fed a standard or a HF/HS diet. In mice, no important changes concerning the measured parameters were recorded among the three phenotypes obtained (Lean not diabetic, Lean Diabetic, Obese diabetic), despite the observation that the HF diet induced insulino-resistance in all animals. In rats, in agreement with our working hypothesis, the two diets (HFr and HF/HS) induced different consequences on mitochondrial activity. In particular, the HFr diet induced: i) insulin resistance in the absence of obesity; ii) a decrease in mitochondrial activity; iii) an increase in ROS (reactive oxygen species) production. By contrast, the insulino-resistant status associated with obesity induced by the HF/HS diet occurred without important changes in mitochondrial activity and ROS production. Interestingly, comparison of data obtained in rats and men demonstrated that the rat model is relevant for the study of the consequences of unbalanced diets in humans. In the two species, a higher mitochondrial activity was associated to an increasing obesity degree, excepted when obesity reached excessive levels in men. Moreover, in the two species, ROS production was higher in the lean than in the obese phenotype, thus suggesting that these molecules could paradoxically exert a preventive influence on the induction of obesity. In all experimental models and in humans, obesity absence seemed to be associated to an increased T4 to T3 conversion, thus favouring lipolysis. Transgenesis induced-changes in mitochondrial activity influenced glucose metabolism differently according to the genetic target. AIF suppression or liver expression of a Malonyl CoA insensitive CPT1 improved insulin sensitivity when mice were grown under a standard diet. In this later experiment, glucose tolerance was also improved under a standard or a HF/HS diet. P43 (mitochondrial T3 receptor) suppression, reducing mitochondrial activity, improved insulin sensitivity and glucose tolerance in mice fed a standard diet. Surprisingly, glucose tolerance was severely impaired using a HF/HS diet despite the maintenance of an improved insulin sensitivity. Therefore, these data demonstrate that a causal relation between mitochondrial activity impairment and the occurrence of different features of a metabolic syndrome could be only established after a very accurate study of different aspects of what is called "mitochondrial activity". Lastly, *in vitro* experiments has allowed us to characterize a set of nuclear genes under the control of the organelle activity. More interesting is the observation that, in mice, study of the expression of some of these genes in muscle or liver allowed us to differentiate obese or diabetic phenotypes in animals fed a HF/HS diet.

[A3]

### **Lipid droplets, mitochondria and cell autophagy in adipose stromal vascular fraction cells**

**Aldona Dembinska-Kiec**, Anna Gruca , Beata Kiec-Wilk, Magdalena Korczyńska; Urszula Czech, Katarzyna Janczarska, Joanna Góralaska; Agnieszka Śliwa; Anna Knapp

*Department of Clinical Biochemistry Collegium Medicum, Jagiellonian University; Cracow, Poland*

Autophagy (autophagocytosis) (AF) is the cellular defence mechanism(s) linking substrate metabolism with longevity, protecting against cellular/tissue malformation. AF is involved in substrate-regulated tissue growth and remodeling, helping to maintain a balance between the

synthesis, degradation, and recycling of damaged cellular organelles ( mitochondria, peroxisomes) and products such as modified proteins, membranes of endoplasmic reticulum (ER) by autophagosome formation. Disturbed AF was found in neurodegenerative (Alzheimer, Huntington), liver, heart, inflammatory diseases, myopathies, diabetes. Chaperone ( HSP90; HSP90 etc) mediated autophagy (CMA) assists the fusion of autophagosome associated proteins with lysosomes via the lysosomal membrane protein type 2A (LAMP-2A) receptor. The ATP synthesis is driven by oxidative phosphorylation (OXFOS) dependent on the mitochondrial electrochemical gradient built across the inner mitochondrial membrane ( $\Delta\Psi$ ) by the Mitochondrial Membrane Permeability Pore system (MMP). This system also controls mitochondrial swelling and oedema and is sensitive to ROS ( generated by starvation, shortage of growth factors, inflammatory cytokines, ER-stress, uncoupling of mitochondria, nitric oxide etc). It has been documented that small decrease of MMP and transient increased mitochondrial swelling is necessary to initiate the protective autophagy process. On the contrary prolonged, potent loss of MMP induces cellular apoptosis, necrosis and death. Dietary PA and OA are known modulators of mitochondrial MMP ( $\Delta\Psi$ ). **Aims:** Since the remodeling of adipose tissue ( differentiation and dedifferentiation ie “jojo-effect” ) is dependent on the substrate supply, gene expression as well as angiogenesis ( endothelial function), the study was undertaken to analyze the influence of different type of dietary FFA on autophagy/apoptosis process in cells of the human adipose tissue ie stromal vascular fraction progenitors (SVF) as well as in differentiated HUVEC cells. **Material and Methods:** Human SVF as well as HUVECs were incubated with non-toxic amounts of FFA (PA, OA, AA, EPA) for 24 hours. To induce the metabolic cellular stress, TNF $\alpha$  (5-25 ng/ml ) the known antagonist of insulin was added for the last 4 hours of incubation. MMP ( $\Delta\Psi$ ) was measured by the *high throughput* immunofluorescent confocal microscopy in vivid cells in BD Pathway 855 Bioimager (Becton-Dickinson) and by flow cytometry. The oxygen consumption was assessed by the Oxygraph-2k; (OROBOROS Instruments, Innsbruck, Austria), when ATP concentration was measured with ATPlite<sup>TM</sup> Luminescence ATP Detection Assay System (Perkin Elmer). (see the parallel JUMC poster in this session). The qRT-PCR analysis of the *Hsp70*, *Hsp90* and *LAMP-2A* genes expression was performed using *GAPDH* as the reference gene. **Results:** The individual effect of the FFA on cellular metabolism is presented in the separate JUMC poster in this session. The incubation with TNF $\alpha$  concentration-dependently as well as with FFA (PA>>OA>EPA>AA) significantly (0.05>p>0.028). inhibited the expression of *Hsp70*, *Hsp90* and *LAMP-2A* genes in SVF cells. Surprisingly the 20-hour preincubation of SVF with FFA prevented the TNF $\alpha$ -induced inhibition of autophagy related genes (specially *LAMP2A*; EPA>OA>>PA; AA less active) even causing their up-regulation in the presence of the higher TNF $\alpha$  concentration ( EPA>>OA,PA>AA). The effects was different in HUVECs. Only the higher concentration of TNF $\alpha$  tended to increase the measured gene expression. FFA alone tended to up-regulate *Hsp90* and *LAMP-2A* but down-regulated *Hsp70*, gene expression. Preincubation with FFA ameliorated the negative effect of TNF $\alpha$ , however the effect was different in the different HUVEC batches and at the higher TNF $\alpha$  concentration even inhibition of the autophagy-related genes was observed.

**Conclusions:** The effect of TNF $\alpha$  and FFA on the autophagy-related gene expression is cell and FFA specific and differ in the lipid storage prone SVF from the proangiogenic HUVEC. In SVF TNF $\alpha$ , PA and AA decreased the measured gene expression. Coexistence of FFA ( mainly EPA but not AA) with proapoptotic TNF $\alpha$  increased the expression of above genes what may promote the protective autophagy process regulating the lipid droplet accumulation. In the proangiogenic HUVEC the  $\Delta\Psi$  decrease by TNF $\alpha$  and FFA (except EPA ) was associated by the weak up-regulation of the autophagy related genes therefore did not protect against the FFA-induced decrease of  $\Delta\Psi$ , ER stress and apoptosis.

*Project supported by the EU FW7 project: “LipidomicNet” No 202272*

## Metabolic Consequences of Tissue-Specific Remodeling of Lipid Metabolism by Dietary Methionine Restriction

**Thomas Gettys**

Pennington Biomedical Research Center, Baton Rouge, Louisiana, USA

Dietary methionine restriction (MR) is a mimetic of chronic calorie restriction in the sense that MR increases rodent longevity, but without food restriction. We report here that MR also persistently increases total energy expenditure (EE) and limits fat deposition despite increasing weight-specific food consumption. In Fischer 344 (F344) rats consuming Control or MR diets for 3, 9 and 20 mo, mean EE was 1.5-fold higher in MR versus Control rats, primarily due to higher EE during the night at all ages. The day-to-night transition produced a 2-fold higher heat increment of feeding (3.0 C vs 1.5 C) in MR versus Controls and an exaggerated increase in respiratory quotient (RQ) to values greater than 1, indicative of the interconversion of glucose to lipid by *de novo* lipogenesis. The simultaneous inhibition of glucose utilization and shift to fat oxidation during the day was also more complete in MR (RQ ~ 0.75) versus Controls (RQ ~ 0.85). Dietary MR produced a rapid and persistent increase in UCP1 expression in brown (BAT) and white adipose tissue (WAT) in conjunction with decreased leptin and increased adiponectin levels in serum, suggesting that remodeling of the metabolic and endocrine function of adipose tissue may have an important role in the overall increase in EE. We conclude that the hyperphagic response to dietary MR is matched to a coordinated increase in uncoupled respiration, suggesting the engagement of a nutrient sensing mechanism which compensates for limited methionine through integrated effects on energy homeostasis.

[A5]

### Fast and food - body mass index and mitochondrial capacity in human skeletal muscle

**Erich Gnaiger**

*Department of General and Transplant Surgery, D. Swarovski Research Laboratory,  
Medical University of Innsbruck, Innrain 66/6, A-6020 Innsbruck, Austria  
Email: erich.gnaiger@i-med.ac.at; Tel : +43 512 504 24623; Fax : +43 512 504 24625*

A tight relationship is described between mitochondrial respiratory capacity of human skeletal muscle and physical fitness, which quantifies the decline of respiratory function as the result of a sedentary life style in the progression towards obesity.<sup>1</sup> Tissue-OXPHOS capacity is the capacity of oxidative phosphorylation in skeletal muscle, which is the product of mitochondrial density and respiratory intensity (structure times function; i.e. mitochondrial marker per tissue mass times OXPHOS capacity per mitochondrial marker). Tissue-OXPHOS capacity per unit wet weight [ $\text{pmol O}_2 \cdot \text{s}^{-1} \cdot \text{mg}^{-1}$ ] is measured directly in permeabilized muscle fibers, and high-resolution respirometry provides a routine approach under physiological conditions (37 °C; Complex I+II substrate combination)<sup>1</sup> with minimal amounts of tissue biopsy (1 to 3 mg wet weight per assay).<sup>2</sup> In healthy subjects varying from athletic to sedentary life styles, tissue-OXPHOS capacity of vastus lateralis increases linearly with maximum aerobic ergometric performance ( $V_{O_{2,max}}$ ) and declines steeply with body mass index (BMI=body mass per body height squared [ $\text{kg}/\text{m}^2$ ]) in the range of 180 to 60  $\text{pmol O}_2 \cdot \text{s}^{-1} \cdot \text{mg}^{-1}$ . The tissue-OXPHOS/BMI relationship spans from world-class endurance athletes and physically active subjects (normal BMI 20-25), overweight individuals (BMI 25-30) with predominantly sedentary life style, to obese patients who are qualified as healthy controls in studies of type 2 diabetes (BMI >30). Total muscle tissue is unchanged or increases rather than decreases with higher BMI, whereas over-proportionally reduced mitochondrial density per muscle mass

explains the loss of aerobic ergometric performance in the sedentary life style (fast food but slow motion) and development of obesity. Mitochondrial quality (coupling and OXPHOS capacity per mitochondrial marker) is largely maintained, does not appear to be a primary defect contributing to obesity as a risk factor for development of type 2 diabetes, and specific mitochondrial injuries accumulate perhaps as a consequence of reduced mitochondrial density and correspondingly low mitochondrial turnover. Based on the tissue-OXPHOS/BMI relationship and integrating known mechanisms responsible for dysregulation of mitochondrial biosynthesis under conditions of chronic low-grade inflammation, low mitochondrial density is a primary risk factor related to a wide range of degenerative diseases, including type 2 diabetes. The health benefits are emphasized of maintaining muscle mitochondrial density high, particularly with progressive age, as achieved by a physically active and nutritionally balanced life style.

<sup>1</sup>Gnaiger E (2009) Capacity of oxidative phosphorylation in human skeletal muscle. New perspectives of mitochondrial physiology. *Int. J. Biochem. Cell Biol.* 41: 1837–1845.

<sup>2</sup>Boushel R, Gnaiger E, Schjerling P, Skovbro M, Kraunsøe R, Dela F (2007) Patients with Type 2 Diabetes have normal mitochondrial function in skeletal muscle. *Diabetologia* 50: 790-796.

[A6]

### **Procyanidins limit insulin secretion by modifying the mitochondrial membrane potential**

Anna Castell-Auví, Pierre Maechler<sup>1</sup>, Lidia Cedó, Víctor Pallarés, Mayte Blay, Montserrat Pinent, **Anna Ardévol**

*Department of Biochemistry and Biotechnology, Universitat Rovira i Virgili, Tarragona, SPAIN.*

<sup>1</sup>*Department of Cell Physiology and Metabolism, Centre Médical Universitaire. Geneva, SWITZERLAND*

Grape-seed derived procyanidins are bioactive compounds with healthy properties that prevent or improve metabolic syndrome related pathologies, but their role in  $\beta$ -pancreatic cells is not clearly defined (1). We induced metabolic syndrome in rats with a cafeteria diet. We then treated them with 2 different GSPE doses for 30 days. At the end of treatment, insulinemia in the GSPE-treated rats was lower than in the cafeteria animals, as were the mRNA levels of insulin in  $\beta$ -cells (2, 3). To evaluate the mechanisms by which procyanidins exert these effects, we treated INS-1 cells for 24 hours with 25 mg GSPE/L and checked several key points that control insulin secretion in  $\beta$ -cells, these being: glucose uptake (2-Deoxy-glucose uptake; glucokinase (GK) and GLUT-2 mRNA), acid citric cycle (citrate synthase mRNA), mitochondrial membrane potential (rhodamine fluorescence, UCP-2 mRNA), ATP cytosolic levels (luminescence detection), plasma membrane potential (Bis-oxonol fluorescence). INS-1  $\beta$ -cells treated with 25 mg GSPE/L for 24 hours showed decreased insulin secretion at basal glucose (2.5 mM) and also at 15 mM glucose, whilst at the same time there was increased cumulated insulin in the cell and lower insulin expression, thus mimicking the effects of GSPE in cafeteria-treated animals. At glucose entry we found increased 2-deoxy-glucose uptake in GSPE-treated cells, although there was decreased expression of GLUT-2 and GK. We did not find any effect on citrate synthase expression, but we found significant effects on the mitochondrial membrane. GSPE treatment increased UCP2 expression and also uncoupled membrane potential, suggesting that ATP synthesis was prevented from coupling to glucose oxidation. Consequently, we found lower ATP cytosolic levels in GSPE treated cells. If higher levels of ATP initiate insulin secretion, then lower ATP levels will depolarize the  $K^+$  channels of the plasma membrane, which would explain the limitations of insulin secretion under high glucose conditions. However, we also found a limited basal secretion. This could be related to the fact that the plasma membrane also

showed a depolarization in GSPE treated cells at basal glucose. To conclude, 25 mg GSPE/L in INS-1 cells for 24 hours limits insulin secretion mainly by uncoupling mitochondrial and plasmatic membrane potentials, these being two key points in the control of insulin secretion. Cumulated insulin in the cell could be one of the mechanisms that limits its own expression.

1. Pinent M et al. Bioactivity of flavonoids on insulin-secreting cell. *Compr Rev Food Sci Food Saf.* 7:299-308, 2008.
2. Montagut G et al. Effects of a grapeseed procyanidin extract (GSPE) on insulin resistance. *J Nutr Biochem.* , 2009
3. Castell A et al. GSPE modify insulin synthesis and secretion in b-cell (Abstract). *Journal of Diabetes* 1, s 1:A271, 2009

[A7]

**Polyphenols from edible plants and their potential to inhibit glucose-induced mitochondrial stress in the nematode *Caenorhabditis elegans***

**Uwe Wenzel**

Molecular Nutrition Research, Justus-Liebig-University of Giessen, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany;

High glucose levels in the blood of humans are a general characteristic of diabetes but slightly enhanced glucose concentrations are found as well in the elderly due to a decline of insulin sensitivity during ageing. A major aim of ageing research is to identify the molecular mechanisms underlying ageing processes and to identify factors that prolongate such processes in order to maintain a good health status. The nematode *Caenorhabditis elegans* serves in this context as a valuable model. *C. elegans* consists of 959 cells and has an average life span of about 17 days, enabling to perform lifespan studies in a manageable time frame. In the present study it is shown that in wild-type *C. elegans* exposed to 100 mM or 250 mM glucose instead of 50 mM glucose in the control the amount of reactive oxygen species (ROS) in mitochondria is dose-dependently increased. Interestingly, these increased ROS-levels are associated with extended lifespan and increased activities of the antioxidative enzyme catalase, a phenomenon referred to as Mitohormesis. However, when the same treatment was performed with *mev-1* mutants that are characterized by high rates of basal mitochondrial ROS production due to a defect in the respiratory chain, lifespan in this short-lived mutant was even more reduced in spite of similar adaptation responses as observed in the wildtype. Accordingly, it was suggested that in *C. elegans* with high initial ROS-levels additional production of ROS in combination with a saturated antioxidative response inevitably leads to premature death. In agreement with this concept, scavenging of ROS by 100 µM ascorbic acid was able to prevent the lifeshortening effect of glucose in *mev-1*.

Polyphenols, are secondary plant compounds, which possess good ROS-scavenging activities as well. In an experimental setup at 37°C at which lifespans were reduced to 12-15 h, 10 mM glucose caused a further significant reduction of lifespan in both wildtype and *mev-1* animals. Under these stressful conditions ascorbic acid was no longer able to rescue the normal lifespan. However, a number of polyphenols prevented the lifespan reduction completely and RNA-interference for *sir-2.1* showed this factor to be essential for the protection from glucose toxicity. Additional experiments showed that glucose increased fat accumulation whereas a diminished fat depot was caused by activation of SIR-2.1. Accumulation of fat, however, was not involved in lifespan reduction as nematodes in which *pod-2*, the homologue of acetyl-CoA carboxylase, was silenced were slim but still sensitive to glucose and polyphenols with regard to lifespan affection.

In conclusion, in the absence of exogenous stress glucose-induced mitochondrial ROS-generation is associated with Mitohormesis in wildtype *C. elegans* whereas mutants with initially higher rates of ROS respond with an early die-off. Under stressfull conditions, wildtype animals are short-lived in the presence of high glucose concentrations also, which is prevented by polyphenols through activation of SIR-2.1.

[A8]

**Hypoxia signalling in the adipose tissue of individuals with the Chronic Obstructive Pulmonary Disease: Insulin sensitivity and adaptation of adipose tissue to systemic hypoxemia in COPD**

**Ukropec, J**<sup>1</sup>, P. Skyba<sup>2</sup>, P. Pobeha<sup>2</sup>, T. Kurdiová<sup>1</sup>, P. Joppa<sup>2</sup>, I. Klimes<sup>1</sup>, I. Tkac<sup>3</sup>, J. Ukropcova<sup>1</sup>, D. Gasperikova<sup>1</sup>, R. Tkacova<sup>2</sup>

<sup>1</sup> Institute of Experimental Endocrinology Slovak Academy of Sciences, Bratislava, Slovakia

<sup>2</sup> Department of Respiratory Medicine and <sup>3</sup> 4th Department of Internal Medicine, Faculty of Medicine,

P.J. Safarik University and L Pasteur Teaching Hospital, Kosice, Slovakia

**Introduction:** Recent advances expanded our understanding to the pathophysiology of adipose tissue in obesity highlighting importance of the endocrine, metabolic, adipogenic, angiogenic and inflammatory processes. Chronic obstructive pulmonary disease is associated with systemic hypoxemia which to a certain degree translates into tissue hypoxia. It is hypothesized that adipose tissue hypoxia contributes to the pathogenic phenotype of AT in obesity. How could systemic hypoxemia contribute to this phenomenon? **Aim** of this study was to investigate the hypoxia related changes in adipose tissue and the whole body metabolic phenotype in cachectic, lean and obese individuals with COPD. **Methods:** Study population was composed of cachectic (n=15, BMI<21 kg.m<sup>-2</sup>, 62.4±1.9 yrs., SaO<sub>2</sub>:88.4±3.2%), lean-overweight (n=20, BMI 21-30 kg.m<sup>-2</sup>, 63.5±1.5 yrs., SaO<sub>2</sub>:94.1±0.7%) and obese (n=11, BMI>30 kg.m<sup>-2</sup>, 60.2±2.4 yrs., SaO<sub>2</sub>:93.8±0.5%) patients with stable COPD. Samples of subcutaneous adipose tissue (ScAT) were obtained by needle biopsy. Gene expression was determined by the qtRT-PCR. Insulin sensitivity (IS) was measured by euglycemic hyperinsulinemic clamp and fat cell size (FCS) by histomorphometry **Results:** IS was increased in cachectic and decreased in obese patients (p<0.0001). IS was positively associated with the ScAT expression of GLUT4 (R<sup>2</sup>=0.42, p<0.0001) and negatively with leptin (R<sup>2</sup>=0.61, p<0.0001) and proinflammatory markers (IL6, R<sup>2</sup>=0.43; CD68, R<sup>2</sup>=0.36, p<0.0001). Cachexia was associated with the presence of adipocyte atrophy and ScAT of cachectic patients expressed increased levels hypoxia responsive transcription factors ARNT2 (p<0.0001) and HIF3α (p<0.01) as well as that of VEGF (P<0.05), and PPARα (P<0.0001) indicating increased angiogenic response to hypoxia and concomitantly increased mitochondrial fatty acid oxidation. Systemic hypoxemia was represented by decreased pO<sub>2</sub>. PO<sub>2</sub> negatively correlated with the adipose tissue expressions of HIF3α (R<sup>2</sup>=0.16, p<0.007) and VEGF (R<sup>2</sup>=0.16, p<0.009). **Conclusion:** Our data indicate that adipose tissue of cachectic individuals with COPD display increased angiogenic response to hypoxia, which together with the absence of local adipose tissue inflammation markers and the presence of metabolic activation is associated with insulin sensitive phenotype. On the other hand, lack of angiogenic response to hypoxia associated with adipocyte hypertrophy and inflammation is paralleled by whole body insulin resistance in obese COPD patients.

Grant support VEGA 28/7110/27, VEGA 2/7111/27, APVV0122/06, COST FA0602

[A9]

**Muscle mitochondria uncoupling leads to healthy ageing in mice**

**Susanne Klaus**, Susanne Keipert, Anja Voigt

German Institute of Human Nutrition, Potsdam-Rehbruecke, Germany

Introduction: HSA-mUCP1 transgenic mice with ectopic expression of uncoupling protein 1 (UCP1) in skeletal muscle mitochondria show a phenotype of increased body weight, increased energy expenditure, delayed development of diet induced obesity and improved glucose tolerance [1,2]. ROS production is decreased in isolated muscle mitochondria of HSA-mUCP1 mice [3]. Here we investigated the effect of UCP1 expression in skeletal muscle on lifespan, obesity, and glucose homeostasis in relation to macro-nutrient intake in transgenic HSA-UCP mice.

Methods: From 12 weeks of age, male and female wildtype (WT) and transgenic (TG) mice were fed ad libitum with semi-synthetic control diet (HCLF, 41:42:17, 15.5 kJ/g) or two different high fat diets (HCHF, 41:16:43, 17.7 kJ/g; LCHF, 11:45:44, 17.5 kJ/g; all ratios correspond to energy% carbohydrate:protein:fat). We measured body composition, lifespan and insulin sensitivity. At specific time points mice were killed for analysis of substrate metabolism and oxidative stress parameters.

Results: There were diet, gender, and transgene specific effects on weight gain, body composition, life span and insulin sensitivity. Compared to HCLF and LCHF, HCHF feeding rapidly and significantly increased body fat content in WT reaching a maximum between 50 and 70 weeks of age and declining thereafter. Maximum body fat content was higher in females than in males. High fat diets significantly reduced mean lifespan of WT (HCLF: 800 days, HCHF: 560 days, LCHF: 660 days,  $p < 0.0001$ ). Insulin sensitivity decreased with age and to a larger extent on HCHF than on the other diets in WT. HSA-mUCP1 mice had decreased lean mass and body fat mass compared to WT and showed a delayed development of obesity on HCHF. Compared to WT, they showed increased insulin sensitivity and lower triglyceride levels in liver irrespective of the diet. In all groups muscle aconitase activity was increased in WT compared to TG suggesting decreased oxidative stress in TG muscle. Median lifespan of TG was not affected by the diet. Maximum lifespan was not different between WT and TG, but on the two high fat diets mean lifespan was significantly increased in TG compared to WT (LCHF +22%, HCHF +30%).

Conclusion: Skeletal muscle mitochondrial uncoupling leads to a “healthy” ageing phenotype with delayed onset of diet induced obesity and preserving insulin sensitivity. Thereby it alleviates high fat diet induced morbidity and mortality in mice.

- 1) Klaus et al. (2005) *Physiol. Genomics*. 21:193-200.
- 2) Katterle et al. (2008) *Physiol. Genomics* 32:352-359
- 3) Keipert et al (2010) *BBA-Bioenergetics* 1797:324-330

This study is funded by the German Research Foundation (DFG) and the EU (BIOCLAIMS)

[A10]

### **Mitochondrial mutations in the human colon during ageing**

---

**John C. Mathers**

Human Nutrition Research Centre, Institute for Ageing and Health, Newcastle University, Framlington Place, Newcastle on Tyne NE2 4HH, UK

The ageing phenotype results from the accumulation of damaged macromolecules in cells and tissues and this damage also increases susceptibility to age-related diseases. Oxidative damage caused by free radicals is a prominent candidate for such damage and the mitochondria are a likely target for free radical-induced damage as well as being a major source of free radicals.

To investigate the mitochondrial ageing phenotype in human tissue we studied the human colorectal epithelium. The architecture of this epithelium is well described with multipotent stem cells located at the base of the crypts and the progeny of these stem cells populate the crypt. We examined

mucosal biopsy samples from over 200 subjects (aged 17 to 84 years) in whom no bowel pathology was detected at endoscopy. Biopsies were collected from the same anatomical site (10 cm from the anal verge) in each participant. We observed an exponential increase with age in the frequency of crypts exhibiting respiratory chain (RC) deficiency so that by the 9<sup>th</sup> decade of life, about 25% of crypts were RC deficient. In contrast, in a sub-set of subjects aged 45-77 years, we observed little difference in mtDNA mutational load across the age range using the random mutation capture assay. We have begun to examine the functional consequences of RC deficiency by investigating relationships with cell proliferation, apoptosis and crypt size. Proliferation index was significantly ( $P<0.001$ ) lower and there was a tendency for apoptotic frequency to be higher in RC deficient than in normal crypts. Perhaps as a result of these two effects, RC deficient crypts contained significantly ( $P<0.0001$ ) fewer cells than normal crypts. We have good evidence that the frequency of mtDNA mutations increases with age in the human colon but the contribution which this makes to the ageing phenotype or to risk of age-related diseases such as bowel cancer remains to be determined.

**Acknowledgements:** My research in this area is a collaboration with DM Turnbull and LC Greaves and has been funded by the Food Standards Agency (UK) (N12015) and the Newcastle University Centre for Brain Ageing & Vitality supported by the BBSRC, EPSRC, ESRC and MRC as part of the crossCouncil Lifelong Health and Wellbeing Initiative.

Greaves LC *et al.* (2009) *Aging Cell* **8**, 566-572.

Nooteboom M *et al.* (2010) *Aging Cell* **9**, 96-99.

Greaves LC *et al.* (2010) *Experimental Gerontology* Jan 22. Epub ahead of print [PMID: 20096767]



Stonehaven harbour

## ABSTRACTS DAY TWO [A11 – A15]

[A11]

## Different effects of standardized *Ginkgo biloba* extract on heart and liver mitochondria

Vilma Borutaite, Giedre Baliutyte, Rasa Baniene, Sonata Trumbeckaite, Adolfas Toleikis

Institute for Biomedical Research, Kaunas University of Medicine, Kaunas, Lithuania

The biologically active plant extracts (such as *Ginkgo biloba*, etc.) are widely used as food supplements and pharmacological means for various conditions. The standardized *Ginkgo biloba* extract (GBE) is often recommended for the treatment of impairment of cerebral blood circulation and age-related memory disorders. GBE also has been shown to be promising as an adjuvant therapeutic treatment in diabetics against ischemic myocardial damage. As mitochondria are thought to be involved in pathogenesis of these disorders the beneficial effect of GBE may be associated with actions of its bioactive constituents on mitochondria. In this study, we compared the effects of ethanolic GBE on respiration of isolated rat heart and liver mitochondria. We found that GBE stimulates pyruvate-dependent respiration of heart mitochondria and decreases mitochondrial membrane potential. Uncoupling effect of GBE was found to be due to its protonophoric action and is likely to be mediated by the ATP/ADP-translocator and uncoupling proteins (UCP) as this effect was partially eliminated in the presence of carboxyatractyloside (an inhibitor of ATP/ADP-translocator) and GTP (an inhibitor of UCP). In contrast, GBE did not induce uncoupling of liver mitochondria. In heart mitochondria, maximal, ADP-stimulated respiration was slightly stimulated at low and depressed at higher GBE concentrations and was not relieved by classical uncouplers CCCP or DNP indicating that GBE may inhibit the respiratory chain complexes or the substrate transport. However, Complex IV of the respiratory chain was not inhibited by GBE. Mitochondrial H<sub>2</sub>O<sub>2</sub> generation was attenuated by low concentration of GBE probably due to the mild uncoupling. The data suggest that mild but not severe uncoupling activity of GBE may be important in providing pharmacological protection of cellular functions in pathological situations.

[A12]

## Obscure mechanisms for differential adaptation of mice of identical genetic background to high-fat diet

Jan Kopecky

Department of Adipose Tissue Biology, Institute of Physiology of the Academy of Sciences of the Czech Republic, Videnska 1083, 142 20 Prague, Czech Republic

Contribution of both, a genetic component and environmental factors to the development of obesity is well proven. Laboratory mice are frequently used to characterise the mechanisms underlying development of obesity. Inbred strains of mice differ largely in their susceptibility to obesity (1) providing a possibility to study the significance of a specific genetic background for obesity. The genetic component for the susceptibility to obesity is apparent even under condition promoting lean phenotype (2;3), while it becomes unmasked in full only when animals are fed obesogenic diets. In spite of the highly homogeneous genetic background, in obesity-prone strains, such as the C57BL/6J (B6) mice (1), individual mice largely differ in body weight gain and accretion of body fat in response to a long-term feeding obesogenic high-fat (HF) diet (2-5). This heterogeneity in the response to obesogenic environment could not be explained in full by differences in energy intake among individual mice (2). Either stochastic mechanisms or a stable epigenetic modifications of genes of energy balance could affect the phenotypic variations among the animals, as discussed in depth by Kozak and his colleagues (2). In any case, the differential metabolic adaptation of mice to HF

diet provides an important model to further characterise a molecular basis for the pathogenesis of obesity and associated metabolic disorders (5), i.e. pathogenesis of metabolic syndrome in humans. However, the non-genetic mechanisms, which cause the large variations in HF diet-induced obesity in mice, remain unknown. As demonstrated by the group of Kozak (2), the susceptibility to diet-induced obesity in genetically identical mice is a stable phenotype that can be detected in mice shortly after weaning. Their results suggest that (i) epigenetic mechanisms underly the variable obesity phenotypes present among the male B6 mice fed HF diet; (ii) high body weight gainers are metabolically more efficient in the conversion of caloric energy into body weight and eventually eat more; and (iii) expression of genes of Wnt signalling in adipose tissue could serve as an early and highly predictive marker of propensity to obesity of individual mice. As demonstrated by the group of Thorens (4;5), genetically homogenous B6 mice display differential metabolic response to HF diet-feeding not only with respect to development of obesity but also to development of glucose tolerance, while the obese and diabetic phenotypes could be separated among subgroups of the animals. Thus, when the animals were fed HF diet for 9 months, most of them (~50%) become obese and diabetic, whereas ~12% remained lean and diabetic, and ~12% lean and non-diabetic; and the rest of the mice displayed intermediate phenotype (4;5). These differences were associated with differences in gene expression pattern. Thus, propensity to development of obesity was associated with a strong up-regulation of hepatic lipogenic genes and increased levels of plasma VLDL lipoproteins, and increase in muscle lipoprotein lipase gene expression, while the lean and non-diabetic mice exhibited an additional increase in *eNOS* in liver. These data suggest that high lipogenic activity in the liver predispose mice to obesity and results eventually in large accumulation of body fat, leading also to a relatively high flux of fatty acids from liver to peripheral tissues in obese as compared with lean mice (5). We hypothesise that AMP-activated protein kinase (AMPK) could be involved in the differential setting of the rate of the lipogenic flux in the liver and consequently in the highly heterogeneous induction of obesity by HF diet in mice of identical genetic background. AMPK serves as a sensor of cellular energy levels (6-8) and controls metabolic fluxes, namely partitioning between lipid oxidation and lipogenesis. Thus, activation of AMPK in the liver leads to the inhibition of lipogenesis and stimulation of  $\beta$ -oxidation of fatty acids. AMPK also inhibits hepatic gluconeogenesis and stimulates transport of glucose into muscle cells (9;10). In support of this hypothesis, our unpublished results from experiment using whole-body knock out of the  $\alpha 2$  catalytic subunit of AMPK in mice strongly support the notion that an important part of the physiological role of hepatic AMPK is to limit lipogenic flux when the drive for *de novo* lipogenesis is too high. In spite of the fact that the exact mechanism remains unknown, hepatic lipogenesis represents an emerging control point in setting differential propensity to obesity and associated disorders in mice with identical genetic background. Regarding the key role of hepatic lipogenesis in control of plasma lipids profile, it may be suggested that levels of plasmatic lipids and their fatty acid composition could serve as a predictive marker for susceptibility to development of obesity and metabolic syndrome. This hypothesis is suitable for testing in laboratory mice and the results could be useful in clinical studies.

#### Reference List

1. Surwit,RS, Feinglos,MN, Rodin,J, Sutherland,A, Petro,AE, Opara,EC, Kuhn,CM, Rebuffe-Scrive,M: Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice. *Metabolism* 44:645-651, 1995
2. Koza,RA, Nikonova,L, Hogan,J, Rim,JS, Mendoza,T, Faulk,C, Skaf,J, Kozak,LP: Changes in gene expression foreshadow diet-induced obesity in genetically identical mice. *PLoS.Genet.* 2:e81, 2006
3. Tallman,DL, Noto,AD, Taylor,CG: Low and high fat diets inconsistently induce obesity in C57BL/6J mice and obesity compromises n-3 fatty acid status. *Lipids* 44:577-580, 2009
4. Burcelin,R, Crivelli,V, Dacosta,A, Roy-Tirelli,A, Thorens,B: Heterogeneous metabolic adaptation of C57BL/6J mice to high-fat diet. *Am J Physiol Endocrinol.Metab* 282:E834-E842, 2002

5. deF, V, Neubauer,H, Poussin,C, Farmer,P, Falquet,L, Burcelin,R, Delorenzi,M, Thorens,B: Transcript profiling suggests that differential metabolic adaptation of mice to a high fat diet is associated with changes in liver to muscle lipid fluxes. *J.Biol.Chem.* 279:50743-50753, 2004
6. Yamauchi,T, Kamon,J, Minokoshi,Y, Ito,Y, Waki,H, Uchida,S, Yamashita,S, Noda,M, Kita,S, Ueki,K, Eto,K, Akanuma,Y, Froguel,P, Foufelle,F, Ferre,P, Carling,D, Kimura,S, Nagai,R, Kahn,BB, Kadowaki,T: Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat.Med.* 8:1288-1295, 2002
7. Minokoshi,Y, Kim,YB, Peroni,OD, Fryer,LG, Muller,C, Carling,D, Kahn,BB: Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature* 415:339-343, 2002
8. Zhang,BB, Zhou,G, Li,C: AMPK: an emerging drug target for diabetes and the metabolic syndrome. *Cell Metab* 9:407-416, 2009
9. Barnes,BR, Zierath,JR: Role of AMP--activated protein kinase in the control of glucose homeostasis. *Curr.Mol.Med.* 5:341-348, 2005
10. Carling,D: The AMP-activated protein kinase cascade--a unifying system for energy control. *Trends Biochem.Sci.* 29:18-24, 2004

[A13]

### **Qualitative and quantitative analysis of mitochondrial population in 3T3-L1 adipocytes responding to a mild mitochondrial uncoupling: a mitoproteomic approach**

**T. Arnould**, S. Tejerina, A. Houbion, M.Dieu, E. Delaive, P. Renard, M. Raes and A. De Pauw  
*Laboratory of Biochemistry and Cellular Biology, University of Namur (F.U.N.D.P.), rue de Bruxelles, 61, 5000 Namur, Belgium. Email : [thierry.arnould@fundp.ac.be](mailto:thierry.arnould@fundp.ac.be)*

Mitochondrial dysfunction is known to induce mitochondrial biogenesis in many cell lines but more particularly in muscle cells as part of an adaptative response to mitochondrial activity alteration. Besides, it is now well accepted that mitochondrial dysfunction affects lipid-metabolizing tissues such as hepatocytes and adipocytes and constitutes an interesting approach to develop new therapeutic strategies to fight obesity. Indeed, a mild and chronic mitochondrial uncoupling induces a partial de-differentiation of 3T3-L1 adipocytes characterized by a decrease of triglyceride content similar to the one observed in TNFalpha-induced dedifferentiation in 3T3-L1 cells. However, the impact of mitochondrial uncoupling on mitochondrial population (abundance, activity, composition...) in adipose cells is totally unknown. In this study, the effects of mitochondrial uncoupling triggered by carbonyl cyanide (p-trifluoromethoxy) phenylhydrazone (FCCP) at a low concentration (0.5 microM) in 3T3-L1 adipocytes during 6 days were investigated and compared with the effects triggered by 10 ng/ml TNFalpha on mitochondrial population of 3T3-L1 adipocytes. While a remodeling of mitochondrion was observed in adipocytes treated with the uncoupler or the cytokine for 6 days, no major quantitative modifications into mitochondrial abundance could be found in any of these treatments. The potential qualitative modifications of mitochondrial content were then investigated using a proteomic approach. Mitochondrial protein abundance variations among adipocytes treated or not with either FCCP or TNFalpha for 6 days were analyzed (using fluorescent 2D-DIGE) on highly purified mitochondrial fractions. Mass spectrometry analysis revealed several modifications in the abundance of (soluble) mitochondrial proteins such as a decrease in the abundance of pyruvate carboxylase, or an increase in the abundance of mitochondrial heat shock protein 70 in adipocytes treated with FCCP or TNFalpha. However, many mitochondrial proteins have been identified as specifically and differentially abundant either in FCCP-treated adipocytes or in TNFalpha-treated adipocytes, when compared with control adipocytes. Two subunits of complex I (NADH-ubiquinone oxidoreductase 42, NADH-ubiquinone oxidoreductase 30) and chaperon proteins such as glucose-regulated protein E-like 1 and heat shock protein 9 have been found to be more abundant in mitochondria isolated from adipocytes treated with the uncoupler while the b-subunit of ATPsynthase and manganese superoxide dismutase

(SOD2) are more abundant in mitochondria from adipocytes treated with TNF $\alpha$ . *In silico* analysis of transcription factors predicted to regulate nuclear gene expression encoding mitochondrial proteins identified as more abundant in adipocytes exposed to mitochondrial uncoupling has highlighted a potential role of the anti-adipogenic transcription factor PU.1. In addition, DNA-binding activity of this transcription factor was found to be higher in adipocytes treated with the uncoupler than in control adipocytes. While functional analysis is ongoing, these data suggests a role of PU.1 either in the modifications of mitochondrial composition and/or in triglyceride content decrease induced by mitochondrial uncoupling in 3T3-L1 adipocytes. In conclusion, a mild and chronic mitochondrial uncoupling in 3T3-L1 adipocytes, while not sufficient to induce an increase in mitochondrial biogenesis, induces specific qualitative modifications in mitochondrial protein content.

[A14]

### **Polyphenols and carotenoids - bioavailability and relation to chronic diseases**

**Torsten Bohn**

Polyphenols and carotenoids represent bioactive secondary plant components that have been discussed in relation to the prevention of chronic diseases, including cardiovascular diseases and cancer. The both can act as antioxidants and may impact intracellular signalling and communication between cells, such as via tight junctions. However, in order to be bioactive following ingestion, these compounds have first to be released by the food matrix, to be solubilized or, in case of the carotenoids, micellarized, and then taken up either via passive diffusion or uptake via specific transporters. While many studies have focussed on bioactivity of polyphenol and carotenoid profiles as they occur in plants, limited information is still available on changes during gastro-intestinal digestion, including phase I and phase II metabolites, and factors influencing their absorption, including other dietary compounds.

[A15]

### **Mitochondrial function and premature ageing: Impaired function of mitochondria in tissues from mice expressing defective mitochondrial DNA polymerase**

**Shabalina IG<sup>1</sup>**, Edgar D<sup>2</sup>, Trifunovic A<sup>2,3</sup>, Larsson NG<sup>2,5</sup>, Cannon B<sup>1</sup>, Nedergaard J<sup>1</sup>

<sup>1</sup>*The Wenner-Gren Institute, Stockholm University*, <sup>2</sup>*Division of Metabolic diseases Karolinska Institutet NOVUM, Stockholm, Sweden*; <sup>3</sup>*Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases, University of Cologne, Germany*; <sup>5</sup>*Max Planck Institute for Biology of Ageing, Gleueler Strasse 50a, D-50931 Cologne, Germany*.

MtDNA mutator mice expressing mtDNA polymerase with reduced proofreading activity (D257A point change mutation in the catalytic subunit) exhibit several features of premature aging, such as reduced lifespan, weight loss, reduced fat content, manifestation of alopecia, kyphosis and osteoporosis, anemia and reduced fertility (Trifunovic et al., 2004). Recently, we have shown that the observed phenotype in mtDNA mutator mice is a direct consequence of the accumulation of mtDNA point mutations in protein-coding genes, leading to a decreased assembly of the respiratory chain complexes and thus to respiratory chain dysfunction (Edgar et al., 2009). The aim of the present study was to explore the variety of function of isolated mitochondria from different tissues (heart, liver, skeletal muscle and brown adipose tissue) of mtDNA mutator mice. ADP-stimulated oxygen consumption was lower in liver, heart and skeletal muscle mitochondria, supporting earlier demonstrated lower ATP production rate in these mice. Electron transport chain (ETC) activity (NADH-stimulated oxygen consumption in permeabilised mitochondria) and maximal oxidative

capacity (FCCP-stimulated oxygen consumption in intact mitochondria) were impaired in all tissues analyzed, whereas basal respiration (oligomycin-insensitive) and development of Ca<sup>2+</sup>-induced permeability transition pore were not affected. ROS production stimulated by different substrates (complex I- or II-linked) was lower in mitochondria with defective mtDNA polymerase reflecting low activity of ETC and/or low membrane potential. Nonshivering thermogenesis derived from UCP1 activity in brown-fat mitochondria was also declined, although UCP1 content was preserved. Thus this mouse model may be important for understanding the role of mitochondria in mechanism of ageing processes.