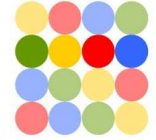


University of Balearic Islands
Laboratory of Molecular Biology,
Nutrition and Biotechnology



mit food

COST Action FA0602: Bioactive food components,
mitochondrial function and health

Workshop and Management Committee Meeting

Mallorca, March 19-20th, 2009



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COST Action FA0602: Bioactive food components, mitochondrial function and health

Mallorca, March 19-20th, 2009

Organization:

Laboratory of Molecular Biology, Nutrition and Biotechnology (LBNB)

University of the Balearic Islands

Prof. Andreu Palou

Dr. Francisca Serra

Dr. Ana M. Rodríguez

Dr. Catalina Picó

Dr. Luisa Bonet

Dr. Paula Oliver

Dr. Joan Ribot

Dr. Juana Sánchez

Dr. Teresa Priego

Ms. Nuria Grandados (Technical Secretary)

Scientific committee:

Prof. Andreu Palou (Chair)

Prof. Jaap Keijer

Dr. Francisca Serra

Dr. Ana M. Rodríguez

Dr. Catalina Picó

Dr. Luisa Bonet

Dr. Paula Oliver

Dr. Joan Ribot

Dr. Juana Sánchez

Dr. Teresa Priego

FINAL PROGRAM

Thursday, March 19th

- 9:15-9:30 **Welcome.** Jaap Keijer (NL) & Andreu Palou (ES)
- 9:30-11:00 Scientific Session I: **Understanding mitochondrial health.** *Chairs: Susan Klaus (DE) & Francisca Serra (ES)*
- Molecular responses to diet-induced mitochondrial stress in *C. elegans*. *Uwe Wenzel (DE)*
 - Retinoblastoma protein, oxidative metabolism and adiposity. *M^a Lluisa Bonet (ES)*
 - Antioxidant status of mitochondria is modulated by nutrients. *Charlotte Lauridsen (DK)*
- 11:00-11:30 Coffee Break
- 11:30-13:00 Scientific Session II: **Studying mitochondrial health.** *Chairs: Barbara Canon (SE) & Catalina Picó (ES)*
- Biogenesis of mitochondrial complex I in health and disease. *Rolf Janssen (NL)*
 - Determination of antioxidant properties of foods. *Atif Can Seydim & Zeynep Guzel-Seydim (TK)*
 - Mouse models of mtDNA defects and their relevance for human disease. *Henna Tynismaa (FI)*
- 13:00-15:00 Lunch
- 15:00-16:30 Scientific Session III: **Studying mitochondrial function.** *Chairs: Johanna Mihaly (HU) & Ana M^a Rodríguez (ES)*
- Influence of mitochondrial DNA level on cellular energy metabolism. *Christophe Rocher (FR)*
 - Gene expression silencing: tracking miRNA in lipid metabolism. *Thierry Arnould (BE)*
 - Detection and identification of bioactive compound in leek before and after processing. *Marc deLoose (BE)*
- 16:30-17:00 **Introduction (short) presentations of new members.** *Chairs: Jaap Keijer (NL) & Joan Ribot (ES)*
- Nutritional and hormonal impact on structural alterations of brown adipocyte mitochondria. *Bato Korac (RS)*
 - Past, presence and future of our research on adipokines, obesity and endocrine disorders. *Jozef Ukropec (SK)*
- 17:00-17:30 Coffee Break
- 17:30-19:00 **Management committee meeting**
- 21:00 Dinner

Friday, March 20th

- 9:00-10:30 Round Table I: **The impact of food components on mitochondrial function.** *Chairs: Rolf Kristian Berge (NO) & Paula Oliver (ES)*
- Mitochondria, adipose failure and bioactive food components. *Jaap Keijer (NL)*
 - Procyanidins interaction with biochemical-physiological processes. *Montserrat Pinent (ES)*
 - Molecular insight on the procyanidins bioactivity.. *Anna Ardévol (ES)*
- 10:30-11:00 Coffee Break
- 11:00-13:00 **Poster Session.** *Chairs: Jan Nedergaard (SE), Teresa Priego (ES) & Joana Sánchez (ES)*
- 13:00-15:00 Lunch
- 15:00-16:30 Round Table II: **Mitochondrial dysfunction.** *Chairs: Lluís Arola (ES) & M^a Lluisa Bonet (ES)*
- Investigating the role of mitochondrial UCP1 in thymus function *Richard Porter (IR)*
 - Mitochondrial dysfunction and adipose tissue pathophysiology: lessons from lipodystrophy. *Francesc Villarroya (ES)*
 - Endothelial and adipose stromal vascular fraction metabolism in the presence of beta-carotene and fatty acids. *Aldona Dembinska (PL)*
- 16:30 **Closure.** Jaap Keijer (NL) & Andreu Palou (ES)

Timing organisation:

- *Scientific Sessions: 20' each presentation + 10' for questions*
- *Round Tables: 20' each presentation and after all presentations a debate will be created*

ORAL PRESENTATIONS

Molecular responses to diet-induced mitochondrial stress in *Caenorhabditis elegans*

Wenzel U., Heidler T., Daniel H.

Molecular Nutrition Research, Justus-Liebig-University Giessen, Heinrich-Buff-Ring 26-32, 35390 Giessen, Germany

Reactive oxygen species (ROS) generated as a by-product of mitochondrial metabolism and respiration are considered to play a pivotal role in aging but also to cause hormetic extension of life span by up-regulating distinct stress-response genes. Using a modified liquid axenic medium we provided high amounts of glucose to wildtype *Caenorhabditis elegans* and the ROS-sensitive mutant *mev-1* and assessed the impact on mitochondrial respiration and ROS-production, and on life span. Both strains responded to the high glucose load with increased respiration and mitochondrial ROS-generation but *mev-1* had much higher initial ROS-levels. Insulin-signaling was activated by glucose as indicated by the reduction in nuclear localization of the Forkhead transcription factor DAF-16. Accordingly, DAF-16 could not participate in any adaptations to glucose-induced ROS by its transcriptional activation of genes important for stress response and longevity. Nevertheless, in wildtype nematodes those adaptations occur as characterized by increased catalase activities which were associated with enhanced lifespan. Although catalase activities were higher after glucose application in *mev-1* as well, premature death was the consequence.

Flavone and resveratrol are secondary plant compounds which also, to a similar extent, increased the levels of mitochondrial ROS but, in contrast to glucose, caused the nuclear translocation of DAF-16. This was associated in wildtype nematodes with an increase in lifespan by resveratrol and a decrease by flavone. An explanation for these divergent results could be the opposite effects of both compounds on the activities of NAD⁺-dependent deacetylases, the sirtuins, that are needed for the transcriptional activity of DAF-16 and that were increased by resveratrol and decreased by flavone.

In conclusion, our studies provide evidence that mitochondrial ROS can prolong lifespan by Mitohormesis whereas beyond a certain threshold they lead to premature death. Moreover, the influence of selected secondary plant compounds on important lifespan regulators, such as sirtuins, appear to overlap the importance of mitochondrial ROS for longevity.

Retinoblastoma protein, oxidative metabolism and adiposity

Bonet¹ M. L., Mercader¹ J., Ribot¹ J., Murano² I., Feddersen³ S., Cinti² S., Madsen^{4,5} L., Kristiansen^{3,5} K., and Palou¹ A.

¹Laboratory of Molecular Biology, Nutrition and Biotechnology, Universitat de les Illes Balears and CIBER Fisiopatología de la Obesidad y Nutrición, Palma de Mallorca, Spain; ²Department of Molecular Pathology and Innovative Therapies, Faculty of Medicine, University of Ancona (Politecnica delle Marche), Ancona, Italy; ³Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark; ⁴National Institute of Nutrition and Seafood Research, Bergen, Norway; ⁵Department of Biology, University of Copenhagen, Denmark.

The retinoblastoma protein (pRb) plays important roles in the control of cell cycle, apoptosis and cell differentiation and, in particular, is involved in the control of adipocyte biology. Both inhibitory and stimulatory effects of pRb on white adipogenesis have been described in cell models. In addition, pRb appears to exert an inhibitory effect specifically on brown adipogenesis, having been proposed to act as a molecular switch between white and brown adipocyte differentiation. Unlike wild-type preadipocytes, preadipocytes lacking pRb differentiate in culture into adipocytes that express brown adipocyte-specific genes and have increased mitochondrial content. Consistent with these results, treatments promoting increased oxidative metabolism in murine white adipocytes are accompanied by a down-regulation of the expression and/or inactivation of the pRb. Moreover, adipose tissue-specific homozygous ablation of the pRb gene in adult mice has been shown to protect against high fat diet-induced diabetes because of increased energy expenditure linked to mitochondrial activation in brown and white adipose tissues. Our recent studies add insight into the physiological relevance of the pRb in the regulation of whole body energy metabolism by revealing similar effects in a more physiological model of generalized, partial deficiency throughout development. Thus, compared with wild-type littermates, germ-line pRb haploinsufficient (Rb+/-) B6 mice were relatively protected against diet-induced obesity, insulin resistance and hepatosteatosis. Compared to wild-type littermates, Rb+/- mice fed a high fat diet displayed higher expression of peroxisome proliferator-activated receptor α as well as of genes involved in mitochondrial function, cAMP sensitivity, brown adipocyte determination and tissue vascularization in white adipose tissue depots. Overall, findings support a role for pRb in modulating energy metabolism and the plasticity of the adipose tissues in vivo.

Antioxidant status of mitochondria is modulated by nutrients

Lauridsen C.

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Lipid oxidation is of major importance for quality deterioration in meat and meat products, but is also of concern with regard to the quality of feed. In addition, there has been a growing desire over the last years to maintain by dietary means the balance between prooxidants and antioxidants in the live animals, since a number of important production diseases in farm animals are thought to be associated with oxidative stress. Lipid oxidation is primarily initiated in the unsaturated fatty acids of the phospholipids, which are an integral part of mitochondrial and microsomal membranes. Oxidation of polyunsaturated fatty acids present in cell membranes may lead to cell injury due to disruption of the normal membrane structure and function. The dietary intake of unsaturated fat is, therefore, known to increase the requirement for vitamin E, which is a membrane-associated antioxidant that effectively protects the organism against free radical species capable of initiating and propagating lipid oxidation. However, vitamin E does not function as the only antioxidant *in vivo*; it is an integrated party of the network of antioxidant (e.g. superoxide dismutase, glutathione peroxidase, and vitamin C). In experiments with chickens and pigs we have investigated the effect of feeding supranutritional levels of vitamin E in combination with different levels of prooxidants and fatty acid composition on the antioxidant status of mitochondria and microsomes in red and light muscles. In conclusion, incorporation of α -tocopherol into the membranes can be enhanced by dietary manipulation, and the dietary vitamin E supplementation increased the antioxidant status. Muscle α -tocopherol levels appeared to be a dominant factor in determining the oxidative stability of muscle mitochondria and microsomes rather than fatty acid composition.

Biogenesis of mitochondrial complex I in health and disease

Janssen R.

Wageningen University. Netherland.

Oxidative phosphorylation (OXPHOS) is the final biochemical pathway of ATP production in the cell. Defects of the OXPHOS system are amongst the most frequent inborn errors of metabolism and result in many, often devastating diseases affecting different organs and tissues. The OXPHOS system consists of five multiprotein complexes that are built out of 89 protein components, which are encoded by both the mitochondrial and nuclear DNA. Correct biogenesis and functioning of the OXPHOS system is dependent on the finely tuned interaction between the nuclear and the mitochondrial genomes. Disturbances of the system can be caused by numerous genetic defects and can manifest in a variety of metabolic alterations that result in a plethora of clinical symptoms. The most common cause of OXPHOS disease is complex I deficiency. Complex I is the largest and most complicated of five complexes and represents the main entrance of the OXPHOS pathway. The biogenesis of complex I is an intricate process of assembly of 45 individual subunits into a membrane-bound multiprotein structure. This process is suspected of being facilitated by the action of several assembly factors.

Using 2-dimensional blue native-polyacrylamide gel electrophoresis and RNA interference we identified human complex I assembly factors, NDUFAF1 and Ecsit, characterized their functions in the assembly process, and investigated their involvement in complex I deficiency. By means of a baculoviral complementation assay, we showed that lack of a third complex I assembly factor, NDUFAF2, resulted in disturbance of complex I assembly and alterations in mitochondrial physiology, and is the cause of complex I deficiency in a unique case of a contiguous gene deletion. The proposed roles of assembly factors NDUFAF1, NDUFAF2 and Ecsit are integrated in a revised model of the complex I assembly pathway.

Determination of antioxidant properties of foods

Guzel-Seydim Z., Seydim A. C., Budak N.

Suleyman Demirel University, Department of Food Engineering, Isparta, Turkey

It is necessary to determine the efficacy of natural antioxidants in foods; the antioxidant assays are mainly classified to two groups as inhibition assays and assays involving the presence of antioxidant systems. Especially ORAC (Oxygen radical Absorbance Capacity), TEAC (Trolox Equivalent Antioxidant Capacity)/ABTS (2,2-azinobis(3-ethylbenzothiazoline-6-Sulphonic acid), FRAP (Ferric reducing ability of plasma), TRAP (Total radical Trapping Antioxidant Parameter) and DPPH (2,3-diphenyl-1-picrylhydrazyl) assays are mainly used for the determination of antioxidant properties of food samples. The TEAC assay is based --on the suppression of the absorbance of radical cations of ABTS by antioxidants in the test sample. Recently, ORAC assay is widely accepted and fluorescent probe, fluorescein is used to determine free radical damage; it is based on the inhibition of the peroxy-radical-induced oxidation initiated by thermal decomposition of azo-compounds such as [2,2'-azobis(2-amidino-propane) dihydrochloride (AAPH)]. Total phenolic content of foods is usually correlated with antioxidant assays. Protein content of foods is also important since proteins have a capacity to inhibit lipid oxidation which can contribute to the antioxidant activity of foods.

Total phenolic content, ORAC and TEAC assays were used to measure antioxidant activity in various food samples. Apple, grape, pomegranate juices, different type of wines, vinegars, chocolate milk, strawberry milk, plain and fruit yogurt, some traditional foods like pomegranate sour and "şalgam" (fermented red carrot juice), rose bud jelly and rose syrup samples were evaluated. The results differ widely; for example, antioxidant activity of samples was significantly increased after maceration during red wine production. In vinegar samples, results were ranged between 4,30- 14,5 mM trolox equivalent antioxidant activity. Milk has some phenolic content depending on the type of animal feeding and composition of amino acids. Especially, fruit pure added dairy products had higher antioxidant properties. It is known that antioxidant capacities of foods are very important for health; research on antioxidant status of foods will provide significant information not only for scientific community but also for consumers.

Mouse models of mtDNA defects and their relevance for human disease

Tyynismaa H., Suomalainen A.

Biomedicum Helsinki, Research Program of Molecular Neurology, University of Helsinki, and Department of Neurology, Helsinki University Central Hospital, Helsinki, Finland.

Qualitative and quantitative changes in mitochondrial DNA have been shown to be common causes of inherited neurodegenerative and muscular diseases, and have also been implicated in aging. These diseases can be caused by primary mtDNA mutations, or by defects in nuclear-encoded mtDNA maintenance proteins that cause secondary mtDNA mutagenesis or instability. Furthermore, mtDNA copy number has been suggested to affect cellular tolerance to environmental stress. The mechanisms that regulate mtDNA copy number and the tissue-specific consequences of mtDNA mutations are still largely unknown. As post-mitotic tissues differ greatly in their need for mtDNA maintenance from proliferating cultured cells, and as most mitochondrial diseases affect post-mitotic cell types, the mouse is an important model to study mtDNA defects. Recently developed mouse models and their contribution to our knowledge of mtDNA maintenance and its role in disease will be presented.

Influence of mitochondrial DNA level on cellular energy metabolism

Rocher¹ C., Taanman² J., Pierron¹ D., Faustin¹ B., Benard¹ G., Rossignol¹ R., Malgat¹ M., Pedespan³ L. and Letellier¹ * T.

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The total amount of cellular mitochondrial DNA (mtDNA) varies widely and seems to be related to the nature and metabolic state of tissues and cells in culture. It is not known, however, whether this variation has any significance *in vivo*, and to which extent it regulates energy production. To better understand the importance of the cellular mtDNA level, we studied the influence of a gradual reduction of mtDNA copy number on oxidative phosphorylation in two models: i) a control human cell line treated with different concentrations of 2', 3'-dideoxycytidine, a nucleoside analogue that inhibits mtDNA replication by interfering with mitochondrial DNA polymerase γ , and ii) a cell line derived from a patient presenting mtDNA depletion. The two models were used to construct biochemical and phenotypic threshold curves. Our results show that oxidative phosphorylation activities are under a tight control by the amount of mtDNA in the cell, and that the full complement of mtDNA molecules are necessary to maintain a normal energy production level.

Gene expression silencing: tracking miRNA in lipid metabolism

Arnould T. and Tejerina S.

University of Namur (FUNDP), Belgium

RNA-mediated gene silencing (RNA interference) is a powerful way to knock down gene expression and the endogenous regulation of gene expression by miRNA is now a hot topic in many fields, while still in its infancy in lipid metabolism understanding. We will describe some generalities on miRNA biological aspects regarding expression, maturation and mechanisms of action. We will next cover some aspects of candidates already reported (or predicted/suspected) as major actors in the control of lipid metabolism as well as in the differentiation of adipocytes. Finally, we will address a still ongoing research in which we tried to identify miRNA in adipocytes responding to mitochondrial dysfunction triggered by either a chemical uncoupler (FCCP) or TNF α . In this context, we will evidence the role of PPAR γ ligands in their expression but also the difficulties to assess functions of miRNA candidates by the identification of experimentally validated putative target genes.

Detection and identification of bioactive compound in leek before and after processing

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The aim of the project is to support horticulture production in Flanders and second to generate knowledge that motivate the consumer to enhance his daily intake of vegetables. Leek (*Allium ampeloprasum* var. *porrum*) has been chosen as a model crop as leek is in Flanders one of the most important crop cultivated outdoors. Leek and its consumption has a rather old fashioned and traditional image. This opens space for introduction of innovations.

It is well known that a diet rich in fruit and vegetable content can reduce the risk of chronic disease, but the traits or products responsible for the beneficial human health effects of vegetable and fruit consumption are not well known. Important is also to consider the impact of processing of the vegetables on the bio availability of the bio active compounds. If we want to reach the final goal to improve health by increasing the daily intake of vegetables, then information is needed that can improve the quality of the crops produced, that motivate the consumer and finally processing should take into account the need for convenience and the preservation of the bio availability of the active compounds in the final product.

This research project aims to analyze bioactive compounds like flavonoids (kaempferol, quercetin, ...), organosulfur compounds (alliin, methiin, ...) and inulin. These phytochemicals are known to have a positive influence on health. Beside this specific compounds, also unknown compounds in leek will be determined in function of the genetic diversity, different plant parts, the growth stage and the growth conditions. Finally the influence of processing techniques such as cooking, steaming, fermentation,...on the content of this compounds will be analyzed. This will result in knowledge that may lead to improve processing methods with a maximal preservation of the desired compounds.

To be able to reach this goal it will be important to have access to methods that allow to identify correlations between presence of (bio active) compounds in the food and effects on physiological processes in the human body. The use of mitochondria as a model might be an option for this.

Past, presence and future of our research on adipokines, obesity and endocrine disorders

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The primary target of our research is to study the role of adipose tissue and skeletal muscle in determining metabolic phenotype of the individual in an association with (i) changes in lipid and glucose metabolism (ii) endocrinology of adipose tissue and (iii) tissue hypoxia. Several aspects had been previously studied on rat models of diet-induced obesity and insulin resistance and also in hereditary hypertriglyceridemic and insulin resistant rats. More recently, we adopted many new tools enabling us to start with the clinical and “translational” research. At the moment we do cross-sectional studies on lean, obese and morbidly obese individuals, with the different levels of glucose tolerance. Our patients are metabolically tested with the aid of indirect calorimetry and euglycemic hyperinsulinemic clamp. Fat mass and fat distribution as well as muscle and hepatic lipid content are determined by MRI imaging and spectroscopy. Samples of fat and muscle are being obtained by percutaneous biopsy and used for RNA, DNA and protein isolation and fixed for immunohistology and for electron microscopy (muscle). In addition, adipocytes are routinely separated from the stromal fraction by collagenase digestion. We are also trying to implement different protocols investigating mitochondrial physiology in permeabilized skeletal muscle fibers as well as in isolated adipocytes using the Clarke-type oxygen and TPP+ electrodes. Moreover, we started to work with the primary human skeletal muscle and adipocyte cultures to investigate mechanisms accompanying obesity and development of insulin resistance.

Our research is supported by 7FP-EC LipidomicNET, COST BM0602, COST FA0602

Mitochondria, adipose failure and bioactive food components.

Keijer J.

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Adipose tissue is an essential, highly dynamic and metabolically active tissue that vigorously communicates to support its primary function: the storage of lipids. It performs this function to secure the energy supply of the body and to prevent lipotoxicity. Adipose tissue is essential for maintaining a healthy glucose and lipid homeostasis and failure results in disease. I propose that initial adipose failure following long term excess energy intake is the result of reduced mitochondrial capacity and metabolism. I will present our (collaborative) research on different classes of bioactive food components, fatty acids and polyphenols, they can improve mitochondrial function and consequently prevent adipose dysfunction.

Procyanidins interaction with biochemical-physiological processes

Pinent M.

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Procyanidins are phenolic compounds widely spread in plants and therefore found in human diets through fruits, vegetables, and beverages such as red wine, tea and chocolate. In the context of a society interested in obtaining benefits from food beyond their nutritional values, procyanidins emerge as interesting molecules since they possess several beneficial effects. Our research group therefore is interested in studying the properties of these compounds, and the mechanisms used to exert such effects.

Bioavailability of procyanidins is a central point to consider concerning further interaction of procyanidins with biochemical-physiological processes. In this regard, we have shown that native and metabolized forms of procyanidins are absorbed and can be found in plasma in rats after a procyanidin extract ingestion.

Our group has evidences of procyanidins activity on glucose metabolism, inflammation, body weight gain and other functions. Among them, procyanidins have an interesting role on lipid metabolism. We show using different *in vivo* approaches that procyanidins are hypotriglyceridemic, supporting a beneficial role of procyanidins on health.

Molecular insight on the procyanidins bioactivity

Ardévol A.

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Procyanidins have described several bioactive effects that make them interesting candidates for preventing development of risk factors associated with metabolic syndrome. We have previously described the ability of procyanidins to modulate lipidic metabolism, and at the present we have further clues on its effects on glucose metabolism.

High acute procyanidins doses act as insulinomimetic agents on insulin-lacking systems, i. e. streptozotocin-treated rat and 3T3-L1 adipocytes and L6E9 myotubes. They mimic insulin on its ability to stimulate glucose uptake and glycogen and lipid synthesis by using some of insulin mediators (insulin receptor, PI3K, AKT and GLUT-4). But procyanidins also show remarkable differences to insulin mode of action. These divergences suggested an ability of procyanidins to act preventing or improving disturbed glucose metabolisms situations. In fact, chronic procyanidins treatment showed a slight effect preventing intolerance to glucose on rats and also pointed out some other interesting targets for procyanidins to further be analyzed. In this sense, their implication on mitochondrial functionality has been pointed out.

Investigating the role mitochondrial UCP 1 in thymus function

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Our laboratory has recently discovered the existence of uncoupling protein 1 (UCP 1) in the thymus of rats and mice. We are endeavouring to establish the role of UCP 1 in thymus function. Using wild-type and UCP 1 knock-out mice comparisons, we have preliminary data showing (a) no difference in reactive oxygen species production by thymocytes or (b) no difference in quiescent thymocyte oxygen consumptions rates, however (c) we do see a decreased susceptibility to apoptosis in thymocytes from UCP 1 knock-out mice compared to wild-type controls.. A summary of our findings will be presented.

Mitochondrial dysfunction and adipose tissue pathophysiology: lessons from lipodystrophy.

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Distinct forms of lipodystrophy appear as a consequence of uncommon gene mutations, after anti-retroviral treatment of HIV-1 infected patients and, in some cases, due to unknown causal events. Lipodystrophy in HIV-infected patients is associated with peripheral lipoatrophy, visceral adiposity and, in some cases, dorsal lipomatosis (“buffalo hump”), as well as systemic insulin resistance and hyperlipidaemia. Research on the ethiopathogenesis of the disease revealed the pivotal role of mitochondria in adipose tissue. Several anti-retroviral drugs have as side effect the so-called “mitochondrial toxicity”, mainly due to their inhibitory action on mitochondrial DNA polymerase-gamma. Thus, mitochondrial DNA (mtDNA) depletion appears in adipose tissue, resulting in aberrant gene mtDNA expression and overall disturbances in mitochondriogenesis. These alterations cause local and systemic effects via “retrograde signaling” of mitochondrial events on the expression of nuclear genes encoding proteins that are relevant for adipocyte function, including its capacity to release systemic regulatory proteins (adipokines). However, the role of mtDNA alterations in the specific final outcome of adipose depots is unclear and, for instance, mtDNA depletion appears both in the lipoatrophic subcutaneous adipose tissue and in the enlarged lipomatous dorsal depot. This is reminiscent of the observation that point-mutations in the tRNA-Lys gene of mtDNA cause dorso-cervical fat pad enlargements similar to those occurring in HIV lipodystrophy patients. Further research will be required to establish clear cause-to-effect relationships between mitochondrial dysfunction and white adipose tissue alterations, and the precise mechanisms involved. For this purpose, recently developed rodent models (mice showing lipoatrophy associated with mtDNA depletion due to targeted disruption of mtDNA regulatory nuclear genes) and adipocyte cell culture models in which mtDNA and mitochondrial function were targeted, are likely tools for further establishing the role of mitochondria in adipose tissue. On the other hand, the identification of nutritional agents capable of promoting mitochondrial biogenesis in adipose tissue (i.e. polyunsaturated fatty acids) or to prevent mitochondrial dysfunction (uridine-enriched sugar cane extracts) may lead to undertake nutritional strategies to ameliorate adipose tissue disturbances caused by impaired mitochondrial function.

Endothelial and adipose stromal vascular (SVF) fraction metabolism in the presence of beta-carotene and fatty acids

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Background. Adipose tissue consists of heterogeneous population of cells called stromal vascular fraction (SVF) recently used for the tissue reconstructive therapy in humans. The mechanisms of differentiation of SVF cells has been extensively studied, but the influence of nutrients on energy metabolism, differentiation (angio- versus adipo-) of SVF cells is still poorly understood. Endoplasmic reticulum (ER)-stress, connexin 43 (cx43) mitochondrial translocation cooperate with mitochondrial function in cell survival and protection from apoptosis.

Aim. The aim of the study concentrate on evaluation of the effect of dietary free fatty acids (FFA) as well as the antioxidant beta-carotene (BC) on mitochondrial function determining the and proangiogenic- or propadipogenic path of SVF differentiation.

Methods. HUVEC and human adipose tissue *stromal vascular fraction* (SVF) were cultured in EBM medium (EGM Bullet Kit Clonetics) with non-toxic concentrations of PA, AA, EPA and OA (30µM) (Sigma) and BC (3µM) (Roche) for 24 hours. Mitochondria were isolated from HUVEC or SVF by Mitochondrial Isolation Kit for Cultured Cells (Pierce) with Protease Inhibitor (Halt™), and stained by Mitotracker red CMXRos (Cambrex) according to standard protocol. Change in gene expression was analyzed by microarray (Affymetrix) according to standard protocol, confirmed by qRT-PCR (Opticon Research). Western Blot was used for estimating changes of amount of total and phosphorylated form of cellular or mitochondrial cx43 protein. Metabolic activity of mitochondria was analyzed by ATP production (Roche) and oxygen requirement using Oxygraf 2-K (Oroboros). The changes in mitochondrial inner membrane potential were followed by the fluorescence microscopy imaging in vivid cells (Bioimager BD).

Results. Proangiogenic VEGF, bFGF as well EPA and AA inhibited, when PA promoted differentiation of SVF cells to adipocytes. SVF metabolism measured by consumed oxygen and ATP was higher than HUVEC. FFA as well as BC did not significantly change oxygen consumption, but ATP generation was decreased by PA and OA. Tendency to increase metabolism of lipids in SVF cells by EPA and in HUVEC szs noticed, when glucose metabolism was increased OA in SVF. BC and FFA inhibited cx43 genes in both investigated cell lines but neither induced translocation, mitochondrial membrane potential change nor influence oxygen consumption and ATP generation. Microarray analysis revealed an induction of intracellular substrate transporters by FFA, involvement of genes both in metabolism, angiogenesis as well as ER-shock chaperones protein induction.

Conclusion. The differentiation of SVF to adipocytes is regulated by complex mechanisms involving not only metabolic regulation, but may also activate ER-stress related apoptotic/autophagic pathways.

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POSTERS

GSPE modify insulin-degrading enzyme expression

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**L. Arola is a member of MITOFOOD*

Introduction: Insulin-Degrading Enzyme (IDE) is a ubiquitously expressed zinc metalloprotease that has multiple cellular functions. IDE binds insulin with high affinity and degrades it into several fragments. Previous studies realized by our research group had showed that Grape Seed Procyanidin Extract (GSPE) modulates insulin secretion and inhibits its synthesis. Now, we analyze if GSPE affects insulin degradation in the tissues where this enzyme showed high expression: liver, kidney and mesenteric adipose tissue.

Methods: Wistar female rats were fed with a standard diet or cafeteria diet for 13 weeks. Then, animals fed with cafeteria diet were divided in three groups: cafeteria diet rats and cafeteria diet rats treated with 25 or 50 mg of GSPE/Kg, for 30 days. At the end of the treatment, animals were sacrificed to obtain plasma and tissue samples according to Ethical guidelines. Tissue weights were measured, and IDE gene expression was analyzed by quantitative real-time RT-PCR. All statistical analysis calculations were performed using SPSS software.

Results and Discussion: Cafeteria diet almost doesn't affect IDE expression, only a slight decrease was observed in mesenteric adipose tissue expression. However, total IDE in the animal is significantly increased, mainly due to the rise of mesenteric adipose tissue. It reflects a need of the organism to compensate the increase in insulin synthesis provoked by the cafeteria diet induced insulin resistance. GSPE stimulate IDE expression in a dose-dependent way in the liver. From the point of view of total tissue IDE expression, GSPE increased it only at 50 mg/kg at liver, vs cafeteria. This effect suggests a higher ability to degrade insulin, even though renal IDE decreased, since it is a tiny amount vs liver and mesenteric adipose tissue.

Conclusion: These results point out IDE as a target of GSPE, mainly in the liver. Now, a new study to evaluate its real physiological significance has been opened.

Time course changes of mRNA levels in muscle cells of key genes for mitochondrial lipid oxidation upon fasting in vivo, and under its related signals in vitro

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Skeletal muscle has the capacity to adapt to metabolic demands, as shifting from carbohydrates and lipids to almost exclusively lipids as fuel substrates upon fasting.

We analyzed the time-course response to fasting of key genes involved in lipid oxidation in the mitochondria in male Wistar rats in gastrocnemius (high capacity of shifting from glycolytic to oxidative metabolism) and soleus (highly oxidative) muscles. The feeding conditions studied were: *ad libitum* (control), 4, 8 and 24-hour fasting, and 3-hour refeeding after 8-hour fasting. Differentiated C2C12 myotubes were treated for 0, 1, 3, 8, 12 and 24h with adrenaline, free fatty acids (FFA) and AICAR (an AMP-activated protein kinase –AMPK– activator). Uncoupling protein (UCP) 2 and 3, muscle carnitine palmitoyl transferase (CPTm) and PPARgamma coactivator (PGC) 1 alpha (master regulator for mitochondriogenesis) mRNAs were analysed by RT-qPCR.

PGC1 α (transiently) and UCP3 were soon up-regulated upon fasting in both muscles (decreasing upon refeeding), responding similarly in vitro under FFA and epinephrine treatments; AICAR enhanced PGC1 α transient induction by FFA or epinephrine. The magnitude of elevation of UCP3 and PGC1-alpha mRNA levels was significantly higher in gastrocnemius than in soleus, and UCP2 mRNA levels were only slightly increased with fasting in gastrocnemius. CPTm mRNA levels did not change with fasting or refeeding, but they were slightly increased with FFA in vitro.

PGC1 α stands out as a main factor in the muscle response leading to the metabolic/structural shift to a more lipid oxidative metabolism, where up-regulation of UCP3 is an early key event, soon activated by FFA and adrenergic stimulation, and modulated by AMPK activation. The higher capacity to elevate the expression of pgc1-alpha of gastrocnemius is probably related with its bigger capacity, as compared to soleus, to shift metabolism from mixed type to more oxidative, and also in accordance with the later higher elevation of UCP3 mRNA levels, and the slightly elevation of UCP2 mRNA levels.

Mitochondrial stress signalling in human osteosarcoma cells; effects of selenium

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Defects of mitochondria contribute to arise of mitochondrial stress signalling. Chronic mitochondrial stress is implicated in ageing and growing number of human diseases including cardiovascular and neurodegenerative disorders, carcinogenesis and mitochondrial diseases.

We used human osteosarcoma cells (depleted of mtDNA -Rho0 and cybrid with 98% heteroplasmy NARP) to study the effect of chronic mitochondrial stress on cellular energy metabolism, mitochondrial biogenesis and mitochondrial retrograde signaling. We showed that mitochondrial stress induced changes of mitochondrial organisation, the level of some proteins of respiratory chain and mitochondrial membrane potential. It seems that mitochondrial stress signalling involves alternating Ca^{2+} and/or ROS homeostasis. In this cells the signals derived from mitochondria induced activation of nuclear genes like as NRF1, 2 and TFAM.

Our understanding of pathogenesis of mitochondrial diseases is poor and effective therapeutic strategies are not available, therefore we studied effects of selenium supplementation. Selenium is an essential component of several antioxidant enzymes and has been linked to regulatory functions in cell growth and death. We found that selenium supplementation altered of basic cytosolic Ca^{2+} and mitochondrial ROS level, and that had an effect on mitochondrial biogenesis and retrograde signalling.

This investigation enhanced our understanding of mitochondrial diseases pathology and suggested selenium as a potential therapeutic agent.

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Adiponutrin reflects an alteration in the response to feeding conditions related to different types of overweight/obesity

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Different data of our group indicate that nutritional regulation of different adipose specific products involved in body weight maintenance, as for example UCP1 and leptin, is impaired in obese animals. Here we were interested in studying how the obese status affected nutritional regulation of adiponutrin, a protein mainly expressed in the adipose tissue which is highly regulated by feeding conditions in lean animals. Although the precise role of adiponutrin is yet unknown, it has been proposed to be involved in the maintaining of energy homeostasis, probably through lipogenic processes.

We have studied adiponutrin nutritional regulation in animals with different types of overweight/obesity: Wistar rats fed a high-fat diet or a cafeteria diet, compared with normoweight Wistar rats; and obese Zucker rats compared to their lean controls. All animals were submitted to different feeding conditions: feeding, 14-h fasting and 3-h refeeding after fasting. Adiponutrin expression was determined in different adipose depots by real-time PCR.

As expected, in normoweight rats adiponutrin expression decreased with fasting, while levels found in fed animals were re-covered with 3-h refeeding. However, this nutritional regulation was impaired in animals with increased body weight. In Wistar rats fed with a hyperlipidic diet (11% of overweight), the decrease with fasting was lower than in normoweight animals (48% vs 68%) and was not observed in all the depots; while in cafeteria-fed Wistar rats (29% of overweight) nutritional regulation of adiponutrin was completely lost. In genetically obese Zucker animals (18% of overweight) regulation was also altered, the decrease was not as pronounced as in lean Zucker (58% vs 75%) and, moreover, mRNA levels were not recovered in all the depots with refeeding.

In conclusion, adiponutrin is an acute sensor of feeding conditions but its nutritional regulation is altered in overweight/obese animals, thus reflecting an alteration in the response to feeding in the obese state that seems to be dependent on the type of obesity.

The expression of mitochondrial protein-coding genes involved in energy homeostasis is regulated in response to different feeding conditions in peripheral blood mononuclear cells of Wistar rats

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Peripheral blood mononuclear cells (PBMC) are readily accessible biological material which use for expression studies is increasing because they can be easily and repeatedly collected in sufficient quantities in contrast, for example, to adipose tissue or other tissues related with the maintenance of energy homeostasis as muscle or liver. The aim of this study was to asses the effect of different feeding conditions (*ad libitum* feeding, fasting and re-feeding) on PBMC gene expression of 6-month-old Wistar rats, focusing on the study of genes that codifies for mitochondrial proteins involved in energy homeostasis.

Our results of microarray analysis show that 24 mitochondrial protein-coding genes involved in energy homeostasis were significantly regulated in response to fasting and re-feeding conditions in PBMC (one-way ANOVA, $p < 0.01$). Most of these genes are involved in the electron transport pathway and the mitochondrial ATPase system, such as ATPase inhibitory factor 1 (*Atpif1*), coenzyme Q3 homolog, methyltransferase (*Coq3*) and ATP synthase, H⁺ transporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 1 (*Atp5g1*), showing most of them the same expression pattern, significantly decreasing or not changing in response to fasting and increasing after re-feeding. Moreover, we found that genes that codifies for mitochondrial proteins involved in lipid metabolism with an important role in the transport of long-chain fatty acids across the inner membrane, such as carnitine palmitoyl-transferase 1a (*Cpt1a*), and in the biosynthesis of fatty acids, such as acyl-CoA synthetase long-chain family member 3 (*Acsl3*) and 5 (*Acsl5*), were also regulated in response to different feeding conditions. Interestingly, in PBMC, the majority of these lipid metabolism-related genes showed an expression pattern logical with their lipolytic or lipogenic function, as had been previously described in tissues involved in energy homeostasis regulation such as liver.

In conclusion, the regulation of mitochondrial protein-coding genes in response to different feeding conditions suggests that PBMC could be considered as an appropriate model for studies of energy homeostasis.

Phenolic composition and biological activity of *Origanum vulgare* herb extracts

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Origanum vulgare L. plays a primary role among culinary herbs in world trade. *O. vulgare* is a source of essential oils and phenolic metabolites. Phenolic compounds such as phenolcarboxylic acids and flavonoids constitute one of the most important groups of pharmacologically active substances acting as anti-oxidant, anti-microbial and anti-inflammation tools in cells. The aim of research was to assess qualitative and quantitative composition and variability of phenolics on raw material of spontaneous oregano, as well as to evaluate its biological activity according to antiradical activity of plant extracts and their oxidative phosphorylation effect on rat heart mitochondria *in vitro*. Phenolics were analyzed by optimized HPLC method. An on-line HPLC-DPPH system was performed for identification of radical scavenging compounds using equivalent capacity of standard trolox for quantitative evaluation of antiradical activity of compounds present in extracts. Fourteen phenolic compounds: three phenolcarboxylic acids and eleven flavonoids were identified in *O.vulgare* ethanolic extracts. Rosmarinic acid was the dominant compound. The flavonoid complex was characterized by high variability and dominance of hyperoside, naringin, rutin and astragalgin. Rosmarinic acid together with hyperoside and vitexin were confirmed to be the principal radical scavengers. The antiradical activity highly correlated ($r^2=0.72$) with concentration of principal compounds. The extracts which exposed higher free radicals scavenging capacity were tested for its effect of oxidative phosphorylation function on Wistar rats' hearts mitochondria. In an adequate medium, the plant extracts were added at different concentrations (1.5, 2.5, 5.0, 10.0, 15.0 $\mu\text{L/ml}$). The results showed that extracts had no effect on State 2 respiration rate. The extracts of high concentration (5.0-15.0 $\mu\text{L/ml}$) caused decrease (12-52%) of State 3 respiration rate and decrease of respiratory control ratio in rats' heart mitochondria. Respiratory State 4 was not altered by the increasing concentrations. In conclusion, the extracts of oregano with higher free radicals scavenging capacity not injured the mitochondrial outer and inner membranes but decrease the oxidative phosphorylation.

Opposite effects of beta-carotene and retinoic acid supplementation on adipose tissue thermogenic capacity in ferrets

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Retinoic acid (RA) treatment and vitamin A status influences body adiposity in rodents, with a low status favoring increased fat deposition and reduced expression of uncoupling protein-1 (UCP1) in brown adipose tissue. However, we previously described that the intake of pharmacological doses of beta-carotene (BC) (the main provitamin A carotenoid in mammals) resulted in higher body weight gain in the ferret, an animal model that resembles humans in terms of intestinal BC absorption and metabolism.

The aim of this study was to characterize in this animal model whether the mentioned changes in body weight could be explained by changes in adipose tissue thermogenic capacity. We studied the effects of 6-month supplementation with BC (0.8 and 3.2 mg/kg/day) on adipose tissue morphology and UCP1 expression.

BC supplementation resulted in higher body weight (the high-dose), induced depot- and dose-dependent hypertrophy of white adipocytes, decreased the amount of brown-like multilocular adipocytes in the retroperitoneal depot, and decreased UCP1 content in different fat depots. To ascertain whether BC effects could be mediated by RA, 1 week supplementation with RA (0.25 and 25 mg/kg/day) was also studied. Unlike BC, RA treatment resulted in a slight decrease in adiposity, decreased cell lipid accumulation and increased UCP1 content.

In conclusion, RA, but not BC, may have in the ferret comparable effects to those described in rodents, whereas differences concerning BC and RA treatments may be attributable to the different BC metabolism in this animal model with a lower conversion of BC to RA compared to rodents.

Effect of high-fat diet feeding on leptin receptor expression in white adipose tissue in rats and its relation with mitochondrial fatty acid oxidation capacity

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Leptin can act as an autocrine or paracrine signal to change the rate of synthesis and degradation of lipids. Therefore, changes at the level of the leptin receptor in the adipose tissue can be of great importance in development of obesity and other metabolic disorders.

The aim of the present study was to investigate in male and female rats the effects of high-fat (HF) diet feeding on the expression levels of OB-Rb in different depots of white adipose tissue (WAT), and its relation with mitochondrial fatty acid oxidation capacity. Male and female Wistar rats were fed until the age of 6 months with a normal-fat (NF) or an HF-diet. The weight of three different fat depots (retroperitoneal, mesenteric and inguinal) and the expression levels of OB-Rb, PPAR α and CPT1 in these depots were measured.

HF-diet feeding resulted in an increase in the weight of the different fat depots, the retroperitoneal depot being the one with the greatest increase in both sexes. In this depot, HF-diet feeding resulted in a significant decrease in OB-Rb mRNA levels, more marked in male rats. In the mesenteric depot, the effects of HF-diet feeding on OB-Rb mRNA levels were sex-dependent: they decreased in males rats (associated to a decrease in PPAR α and CPT1 mRNA levels), but increased in female rats. In the inguinal depot, OB-Rb expression was not affected by HF-diet feeding.

These results show that a chronic intake of an HF-diet altered the expression of OB-Rb in WAT in a depot and sex dependent manner. The decreased expression of OB-Rb in the internal depots of male rats under HF-diet feeding, with the resulting decrease in leptin sensitivity, may contribute to accelerate the metabolic impact of HF-diet feeding and can help to explain the higher tendency of males to suffer from obesity-linked disorders under HF-diet conditions.

Expression patterns of genes related with lipolysis and fatty acid oxidation in visceral and subcutaneous adipose depots in rats are linked to their site-specific metabolic features

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White adipose tissue is the main site of energy storage in the form of triacylglycerides, which are mobilized when needed through the lipolysis process to provide fuel for other organs. However, adipose tissue is not a single homogeneous compartment, but rather a tissue with specific regional depots which have different cellular size, structural organization and biological functions. Differential lipid-metabolism gene expression in the different depots is, presumably, responsible of their specific functionalities but only sparse studies on that have been previously reported. The aim of this study was to characterize the expression pattern of selected genes involved in lipid mobilization and fatty acid oxidation in internal (retroperitoneal, mesenteric) and subcutaneous (inguinal) adipose tissue depots in rats and their relation with site-specific morphological- and metabolic-features. Gene expression and morphometric analyses were performed. Results showed that the retroperitoneal depot was the one with the largest adipocyte size and with the lowest DNA content per g tissue, while no differences were found in these parameters when comparing the mesenteric and the inguinal depots. In addition, the expression of lipolysis-related genes (HSL, ATGL) was higher in the retroperitoneal than in the mesenteric and inguinal depots, while the expression of fatty-acid oxidation-related genes (PPAR α , CPT1) was lower in the retroperitoneal depot compared with the mesenteric and the inguinal depots. These differences may have a strong impact on lipid metabolism and mitochondrial function. In particular, these would indicate that internal depots (particularly the retroperitoneal, which also presents the largest adipocyte size) may have a higher lipid mobilization capacity and, in turn, a lower fatty acid oxidation capacity in terms of mRNA expression, compared with other depots. This can explain its higher triacylglyceride turnover rates and its greater mobilization of free fatty acids into blood circulation, which has been related with the adverse metabolic impact of visceral fat accumulation.

all *trans* Retinoic acid induces carnitine palmitoyl transferase-1 in hepatoma cells

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Introduction: We have described that all-*trans* retinoic acid (ATRA) treatment enhances lipid oxidation capacity in liver of intact animals, correlated with decreased lipid content in liver and body adiposity. The objective was to investigate the mechanism of ATRA action on lipid metabolism in human hepatoma cell line HepG2.

Methods: HepG2 cells were treated with different doses of ATRA and retinoid receptor agonists or with vehicle (DMSO) for 24 hours. The mRNA levels for uncoupling protein 2 (UCP2), hepatic isoform of carnitine palmitoyl transferase-1 (CPT1-L), peroxisome proliferators-activated receptor α (PPAR α) and acyl-coenzyme A oxidase 1 (ACOX1) were determined by qRT-PCR, using β -actin as internal control.

Results: The expression of CPT1-L (key step for mitochondrial fatty acid oxidation) was increased in a dose dependent manner in cultured hepatocytes in response to ATRA treatment, as described in the liver of acute ATRA-treated mice. We did not observed significant effects on the expression of other analyzed genes involved in fatty acid oxidation. Moreover, we found that ATRA mediated induction of CPT1-L mRNA seems to be at transcriptional level through preformed retinoid X receptors in the cell, but not through retinoic acid receptor.

Conclusions: These results are compatible with an effect of retinoic acid favouring lipid catabolism in hepatocytes. The knowledge about nutrients (or their derivatives) and its mechanism of action, capable of enhancing oxidative metabolism in liver and other tissues can contribute to new avenues of prevention and treatment of obesity and related disorders.

Supplementation of maternal diet with L-Leu during lactation shows sex-differences in adipose tissue gene expression of offspring

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The objective of this study was to evaluate the effects of maternal diet supplemented with L- leucine on offspring, focusing on the expression of selected genes involved in body weight regulation or associated to mitochondrial function.

Virgin female Wistar rats were mated with males and placed in individual cages under controlled conditions, with free access to water and standard chow diet. At day 1 after delivery, litters were adjusted to 10 pups per dam (five females and five males when possible) and dams matched in two groups (n=6). The control group (C), maintained under the same conditions and, the leucine-treated group (L) which had free access to the same chow but supplemented with 2% of L- leucine from day 1 after delivery. On day 21, two pups (one female and one male) of each litter were sacrificed and mesenteric adipose tissue was rapidly removed, weighed and stored at -80°C for ulterior analysis. Analysis of variance (two-way ANOVA) followed by Student's *t* test was done to assess statistical significances between groups.

No differences in body weight or composition were found between C and L pups during lactation. On day 21, no differences in the weight of mesenteric adipose tissue were either found. Adipose tissue leptin, adiponectin and UCP2 mRNA expression levels were significantly lower in female-offspring of leucine-supplemented dams in comparison with their controls, decreasing a 25.2% ($P=0.029$), a 60.1% ($P=0.001$) and a 40.9% ($P=0.001$), for leptin, adiponectin and UCP2, respectively. Unlike females, no changes in the expression of these three genes were found in males. Resistin and UCP3 mRNA levels were not affected either in female or in male-offspring of leucine-supplemented dams.

In conclusion, maternal dietary supplementation of L-leucine during lactation is able to alter the expression of key genes involved in energy homeostasis in mesenteric adipose tissue, particularly in female offspring. Long term effects of these metabolic adaptations are under further investigation.

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