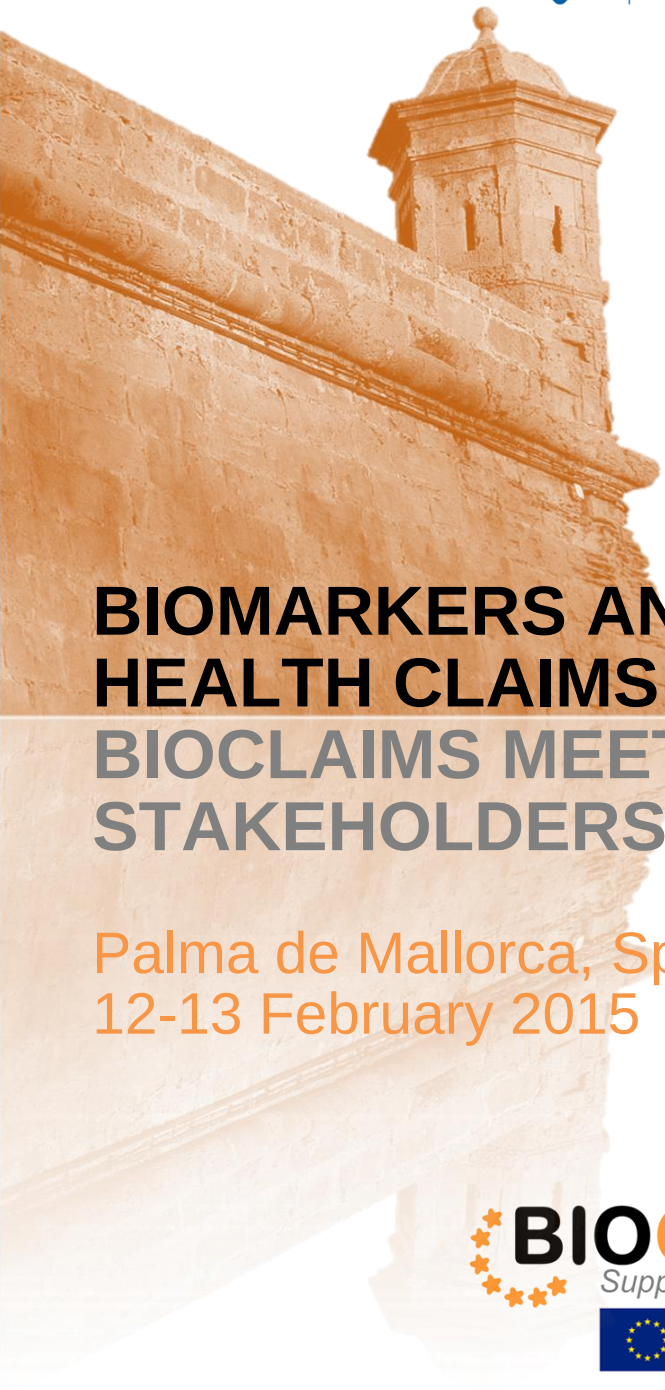

Universitat de les Illes Balears

Laboratori de Biologia Molecular, Nutrició i Biotecnologia

# BIOMARKERS AND HEALTH CLAIMS ON FOOD

## BIOCLAIMS MEETING WITH STAKEHOLDERS

Palma de Mallorca, Spain
12-13 February 2015

BIOCLAIMS.eu
*Supporting health claims on food*

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under agreement nº 244995

## Who has interest to attend?

Representatives of food industry companies
SMEs in the field of food-health
Policy regulators
Marketing specialists on f ood
Academics

“BIOMARKERS AND HEALTH CLAIMS ON FOOD”

ISBN: 978-84-606-5699-9

Edited by: *Joan Ribot, M. Luisa Bonet, Nuria Granados, Paula Oliver, Mariona Palou, Catalina Picó, Pere Josep Pons, Andreu Palou*

Printed in Palma de Mallorca, 2015

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*Ben van Ommen*[6]
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BIOCLAIMS is the first European large collaborative R+D project (Grant agreement no. 244995) addressing the search for new biomarkers as the basis for future health claims made on food, a main bottle neck for expanding the food-health development.

The food and health relationship focuses on maintenance of optimal health, both in terms of physiology and new European legislation (Regulation EC Nr. 1924/2006). Yet, most accepted biomarkers quantify disease endpoints or damage. This has led to major problems in demonstrating health benefits and establishing health claims, and blocks competitive economic and health developments in the food sector.

BIOCLAIMS develops new biomarkers by exploiting the new concept of “health biomarkers” and associated issues through quantification of the robustness of the homeostatic mechanisms involved in maintaining optimal health, based on the assumption that the ability to maintain homeostasis in a continuously challenged environment and changing physiology is key for healthy ageing. Mechanisms involved are investigated during a series of food interventions in animal models and humans using “predisposed” conditions. Human models of presumed impaired robustness in maintaining metabolic and vascular health (caloric restriction, obesity, a pro-inflammatory genotype, an impaired renal function, or a combination thereof) are employed to study the responses of established and novel biomarkers to the challenging of homeostasis. Both advanced analytical methodology including nutrigenomics tools and “whole body” physiological assessments are going to be exploited to derive a series of new biomarkers.

# PROGRAMME

## Thursday 12th February 2015

08.15-08.45h Registration

08.45-09.00h **Welcome and Inauguration**
*Jaume Carot (Vice-Rector for Research, University of the Balearic Islands, SPAIN) & Andreu Palou (BIOCLAIMS Project Coordinator)*

Session 1: **BIOCLAIMS outcomes: *biomarkers and biological plausibility***

*The session is devoted to the presentation of selected candidate biomarkers and related food-health mechanisms as central outcomes of the BIOCLAIMS project.*

S1-Part I Chairs: *Ben van Ommen (TNO, THE NETHERLANDS) & M. Luisa Bonet (University of the Balearic Islands, SPAIN)*

09.00-09.10h BIOCLAIMS project strategy: Nutrigenomic-based biomarkers
*Andreu Palou (University of the Balearic Islands & CIBERobn, SPAIN)*

09.10-09.30h Challenge tests, health and biomarkers
*Jaap Keijer (Wageningen University, THE NETHERLANDS)*

09.30-09.50h Biomarkers of adipose tissue health and expandability and crosstalk with muscle
*Susanne Klaus (German Institute of Human Nutrition, Potsdam, GERMANY)*

09.50-10.10h Acyl carnitines and amino acids as biomarkers of health
*Jan Kopecky (Academy of Sciences of the Czech Republic, Prague, CZECH REPUBLIC)*

10.10-10.30h Lysophospholipids as biomarkers of trends to dyslipidemia
*Lluís Arola, Josep M. del Bas & Antoni Caimari (CTNS & Universitat Rovira i Virgili, Tarragona, SPAIN)*

10.30-10.50h **Coffee break**

S1-Part II Chairs: *Susanne Klaus (German Institute of Human Nutrition, GERMANY) & Alejandro Cifuentes (Institute of Food Science Research, CSIC-UAM, SPAIN)*

10.50-11.10h Blood cell transcriptomic-based early biomarkers of adverse programming effects on obesity and related risk factors
*Catalina Picó & Andreu Palou (University of the Balearic Islands & CIBERobn, SPAIN)*

11.10-11.30h The key biomarkers of inflammation
*Philip C. Calder (University of Southampton, UK)*

11.30-12.05h Emergence of dicarbonyl stress as a key mediator of metabolic, vascular and renal health decline and related biomarkers revealed in BIOCLAIMS. New findings on Nrf2 regulation
*Paul Thornalley & Naila Rabbani (University of Warwick, Coventry, UK)*

12.05-12.30h The BIOCLAIMS cohort as a resource for biomarker discovery and validation
*Brigitte Winklhofer-Roob & Johannes Roob (Karl Franzens University of Graz & Medical University of Graz , AUSTRIA)*

12.30-12.50h Selected nutrient-sensitive biomarkers of metabolic syndrome
*Beata Kieć-Wilk, Małgorzata Malczewska-Malec & Aldona Dembińska-Kieć (Jagiellonian University, Krakow, POLAND)*

12.50-13.10h Knowledge and data driven development of nutrigenomics based biomarkers of robustness
*Suzan Wopereis (TNO Quality of Life, Zeist, THE NETHERLANDS)*

13.10-13.30h **RECAPITULATION AND DISCUSSION**

Chairs: ***Ben van Ommen*** *(TNO, THE NETHERLANDS)* **&** ***Andreu Palou*** *(University of the Balearic Islands, SPAIN)*

**Lunch time**

Session 2. **Industry challenges & Regulatory aspects**

*This session considers the novelties, pending regulatory topics and controversial issues in relation to health claims and biomarkers, together with the novel industry strategies to enter into the space of food and health, and combines invited speakers from administration EC/member states, industries, and other stakeholders.*

S2-Part I Chairs: ***Andreu Palou*** *(University of the Balearic Islands, SPAIN)* **&** ***Manuel Portero-Otín*** *(University of Lleida, SPAIN)*

15.00-15.20h Authorised Claims in the EU: Status, claims on hold, harmonisation and next steps

*Basil Mathioudakis (Head of the Commission's Nutrition, Food Composition & Information Unit. European Commission, Brussels, BELGIUM)*

15.20-15.40h Botanicals. Harmonization? Alternative approaches on Health Claims bypassing the NHCR?

*Jean Savigny (Senior Partner, Keller and Heckman, Brussels, BELGIUM)*

15.40-16.00h Health Claims & Labelling: Law and Practice or how to combine both Regulations

*Montserrat Prieto (Food Law Department Director of the Spanish Federation of Food and Drink Industries (FIAB), Madrid, SPAIN)*

16.00-16.20h Labelling, advertising and presentation of foods. Compliance and infringements

*Vicente Rodríguez-Fuentes (Chair of European Food Law Association (EFLA), Legal Agrifood Abogados, Sevilla, SPAIN)*

16.20-16.50h **DISCUSION**

16.50-17.10h **Coffee break**

Session 3. **Health claims and biomarkers in EFSA and beyond. Towards Health Claims for population subgroups and individuals**

*This session considers health claims and biomarkers for population subgroups or individuals and other potential tendencies*

S3-Part I Chairs: *Lluís Arola (CTNS, SPAIN) & Mariona Palou (Alimentómica, SPAIN)*

17.10-17.30h Nutrition of Mothers and Young Children in the First 1000 days: Learning's from Human Milk and what to Apply?
*Bernd Stahl (Director Human Milk Research. Danone Nutricia Research, Utrecht, THE NETHERLANDS)*

17.30-17.50h Health claims which might arise in relation to riboflavin use in the lowering of high blood pressure: a novel gene-nutrient interaction
*Sean Strain (Northern Ireland Centre for Food and Health and University of Ulster, UK; Chair of the Working Group on Health Claims, EFSA-NDA Panel)*

17.50-18.10h Health claims and probiotics: what and how?
*Yolanda Sanz (Spanish National Research Council (CSIC) and University of Valencia, SPAIN; Vice-chair of the EFSA-NDA Panel)*

18.10-18.30h Strategies and challenges in fat related health claims // Driving Innovation and Value based on health and sustainability
*Diana Roig (Nutrition and Health Manager, Unilever, SPAIN)*

18.30-19.00h **DISCUSSION**

# Friday 13th February 2015

## Session 4. **Further interaction and beyond**

*This session will try a) first to introduce future trends, and an open-mind perspective on new topics combining health claims with other values and consumer needs and expectations; b) Multiple direct short interactions (Speed dating) between stakeholders will be propitiated*

S4-Part I Chairs: ***Alfredo Martínez*** *(University of Navarra, SPAIN)* ***& Josep Mercader*** *(Alimentómica, SPAIN)*

09.00-09.20h Health Claims: Collaborative Industrial Research
*Jose Antonio Moreno (R&D Project Manager, Grupo ORDESA, SPAIN)*

09.20-09.40h Smart-foods and targeted food supplements
*Francisca Serra (R+D Director, Alimentómica SL, SPAIN)*

09.40-10.00h New strategies on health claims. Replacing food components as basis for health claims
*Eric Grande (Regulatory Affairs Director-Directeur des Affaires Réglementaires, Groupe Lactalis, FRANCE)*

10.00-10.20h **DISCUSSION**

S4-Part II (RESEARCH AND COMMUNICATION FACILITIES): Chairs: ***Brigitte Winklhofer-Roob*** *(Karl Franzens University of Graz, AUSTRIA)* ***& Rosa María Lamuela*** *(University of Barcelona, SPAIN)*

10.20-10.40h CIBER: a new consortium for human biomedical research studies in Spain. Multicenter facilities
*Luzma García-Piqueres (Technology-Transfer Manager at Centro de Investigación Biomédica en Red, Madrid, SPAIN)*

| | |
|---|---|
| 10.40-11.00h | AECOSAN as national EFSA focal point an interface for scientific cooperation<br>*Ana Canals (Advisor of the Spanish Agency for Consumer, Food Safety and Nutrition, AECOSAN)* |
| 11.00-11.20h | Beyond claims on health: "Life style, food, health, beauty and happiness. How to combine all with science in a single message?"<br>*Miguel Mira (Director of Communication and External Relations of the Coca Cola Company in Iberia, Madrid. SPAIN)* |
| 11.20-11.30h | **Short break** |
| 11.30-12.30h | **Speed Dating & Poster Party**<br>Chairs:<br>Naila Rabbani *(University of Warwick, UK)*<br>Jaap Keijer *(Wageningen University, THE NETHERLANDS)*<br>Martin Rossmeisl *(ASCR, CZECH REPUBLIC)*<br>Federico Lara *(Lactalis-Puleva, SPAIN)*<br>Rafael Urrialde *(Coca Cola Company, SPAIN)*<br>M. Luisa Bonet *(University of the Balearic Islands, SPAIN)*<br>Ana M. Rodríguez *(University of the Balearic Islands, SPAIN)*<br>*Posters: The abstracts explaining a range of varied issues (industry presentations, scientific outputs, regulatory aspects, stimulating collaboration, etc.) will be exposed (posters) and discussed in small groups.*<br>*Speed dating (multiple single persons meeting one-on-one in around 5 minutes timed sessions so that singles can assess further whether to have subsequent appointments) will be arranged, combined with the poster exhibition.* |
| | **GOOD-BYE** |
| 12.30-13.00h | **BIOCLAIMS internal recapitulation meeting**<br>(*Restricted to BIOCLAIMS Partners*) |

# SPECIFIC SATELLITE SESSIONS

(*Restricted to CIBEROBN and MARCASALUD Partners*)

## Friday 13th February 2015

Chairs:

*Andreu Palou* *(University of the Balearic Islands, SPAIN)*,
*Paula Oliver* *(University of the Balearic Islands, SPAIN)*
*& Xavier Remesar* *(University of Barcelona, SPAIN)*

13.00-13.30h **CIBEROBN.** Program on "New strategies and biomarkers in the prevention and treatment of obesity and eating disorders".

13.30-14.00h **MARCASALUD.** Spanish Network of Excellence on Nutrigenomic Biomarkers and Health Claims.

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# SPEAKER'S ABSTRACTS

## O-01 BIOCLAIMS project strategy: Nutrigenomic-based biomarkers

A. Palou

*Laboratory of Molecular Biology, Nutrition and Biotechnology, Universitat de les Illes Balears, and CIBER de Fisiopatología de la Obesidad y Nutrición (CIBERobn), Palma de Mallorca, Spain.*

The identification and validation of robust biomarkers is crucial for assessing the potential effectiveness and benefits of health-promoting food compounds. This is the basis for new and competitive economic and health developments in the food sector as covered by the recently harmonized legislation on the emerging 'health claims (HC) made on food' in Europe [1]. For a number of physiological functions affected by food compounds there are no useful biomarkers described and for other functions there is a need for earlier biomarkers. This lack is the main bottleneck for the consolidation and expansion of the health claims-based added values in the food sector. BIOCLAIMS is intending to contribute to cover this lack by searching for new biomarkers and associated issues involved in maintaining optimal health, based on the assumption that the ability to maintain homeostasis in a continuously challenged environment and changing physiology is key for healthy ageing. Both advanced analytical methodology including nutrigenomics tools and physiological assessments have been exploited to derive a series of new candidate biomarkers. In particular, parameters characterizing biological systems have been identified and compared in early and later stages to reveal homeostatic differences before clear health differences are visible and new nutrigenomic early biomarkers have been identified to be able to predict functional differences (e.g.: early loss of function when ageing) later on in life. New types of biomarkers and concerned mechanisms have been investigated during a series of food interventions in animal models, cells systems and humans, using more or less “predisposed” conditions. A selection of them are presented by the partners of BIOCLAIMS to the symposium “BIOMARKERS AND HEALTH CLAIMS ON FOOD: BIOCLAIMS MEETING WITH STAKEHOLDERS”, in Palma de Mallorca (12-13 February 2015)

References:

[1]. EU Regulation (EC) No 1924/2006 of the European Parliament and of the European Council of 20 December 2006 on nutrition and health claims made on foods, Official Journal of the European Union L 12 (2007) 3-18.

Acknowledgements: Supported by European Union's Seventh Framework Program FP7 2007-2013 under grant agreement nº 244995 (BIOCLAIMS Project).

## O-02 Challenge tests, Health and Biomarkers

J. Keijer, L.P.M. Duivenvoorde, E.M. van Schothorst

*Human and Animal Physiology, Wageningen University, Wageningen, The Netherlands*
Contact: jaap.keijer@wur.nl.

Tools to quantify health status are needed to substantiate health improvement by nutrition. Health status is usually determined by single static biomarkers, reflecting differences in steady state conditions. Health can also be defined as being metabolic flexible. Metabolic flexibility can be assessed by challenging homeostasis. This can be done invasively, as is done in the oral glucose tolerance test, but also non-invasively, using indirect calorimetry. Recent Bioclaims data underscore the sensitivity of the challenge test approach. Whereas static biomarkers, such as fasting insulin, glucose or leptin could not discriminate in health status in mice fed isocaloric high fed diets of different lipid composition, challenge tests could (Duivenvoorde *et al* 2015a, submitted).

In the Bioclaims project also a nutrient independent, indirect calorimetry based challenge test was developed. The oxygen restriction (OxR) test examines the metabolic response to mildly reduced levels of oxygen. OxR primarily targets mitochondria, which use 90% of body oxygen. We were able to show that a mild OxR challenge, from 21% $O_2$ (normoxia) to 12% $O_2$ was able to reveal a difference in response between adult and old mice that were otherwise healthy (Duivenvoorde *et al* (2014) J Gerontol A Biol Sci Med Sci doi:10.1093/ gerona/glu027). This further confirmed the challenge test concept. OxR was also applied after short term (5 day) feeding of different diets and showed diet dependent differences in response in various tissues and in serum (Duivenvoorde et al (2014) Pflugers Arch - Eur J Physiol doi:10.1007/s00424-014-1553-8). Mitochondrial biomarkers for the OxR response were identified (Duivenvoorde et al 2015b, submitted). The response of specific molecular and physiological alterations upon OxR can be used as biomarkers to test food products on potential health beneficial effects. We are currently testing whether OxR can be used in humans to assess efficacy of nutrients for oxygen delivery to the muscle.

Acknowledgements: This work was supported by the European Union's Seventh Framework Program FP7 2007-2013 under grant agreement no. 244995 (BIOCLAIMS Project).

## O-03 Biomarkers of adipose tissue health and expandability and crosstalk with muscle.

S. Klaus

*German Institute of Human Nutrition in Potsdam-Rehbrücke (DIfE), Physiology of Energy Metabolism, Potsdam, Germany.*

Within BIOCLAIMS we participated in WP1 dedicated to the identification and characterization of early markers for good health using different mouse models. Our objectives were (i) the identification of biomarkers related to healthy aging in transgenic HSA-UCP1 mice (UCP1-Tg mice), and (ii) validation of biomarkers in a dietary intervention and challenge model.

UCP1-Tg mice with ectopic expression of the uncoupling protein 1 in skeletal muscle (SM) mitochondria display a healthy metabolic phenotype linked to the induction of ”browning”, i.e. increased substrate metabolism within typical white fat depots. This is induced by endocrine acting fibroblast growth factor 21 (FGF21) which is secreted from SM of UCP1-Tg mice as part of a stress induced metabolic remodeling program. Additionally, overall lifespan in mice was found to be negatively correlated with the velocity of overall body fat increase. Together this suggests that adipose tissue expansion and function is a critical marker of metabolic health.

We next aimed to evaluate the predictability of short term (5 days) changes in epididymal white adipose tissue (eWAT) gene expression for long term (12 weeks) changes induced by high fat diet (HFD) feeding using a systematic gene profiling approach in mice. We identified 549 genes which were regulated by HFD at both time points, all in the same direction and highly correlated ($r^2$=0.90) between the time-points. Of these, 79% were down-regulated and 21% were up-regulated by HFD. For further validation we then used a 5 day HFD challenge with different nutritional anti-obesity interventions (EGCG supplementation, PUFA supplementation, and increased dietary protein, respectively). Gene expression of Leptin and Mest (mesoderm specific transcript) could be validated as rapidly induced markers of adipose tissue expansion normalized by different anti-obesity interventions. They can thus be useful in short term HFD feeding study protocols in mice for testing and screening of potential anti-obesity food compounds.

Acknowledgements: Supported by European Union's Seventh Framework Program FP7 2007-2013 under grant agreement nº 244995 (BIOCLAIMS Project).

## O-04 Acylcarnitines and amino acids as biomarkers of health

J. Kopecky, O. Horakova, K. Bardova, A. Gardlo, O. Kuda, M. Cerna, I. Brabcova, J. Hansikova, P. Flachs, M. Rossmeisl

*Department of Adipose Tissue Biology, Institute of Physiology Academy of Sciences of the Czech Republic v.v.i., Prague, Czech Republic.*

Obesity and associated metabolic disorders – metabolic syndrome - represent a challenge for the studies of biomarkers of health. Targeted metabolomic analysis of acylcarnitine (AC) and amino acid (AA) profile in plasma is a well-established approach for biochemical screening of inherited metabolic disorders. In this respect, we focused on exploration a potential of targeted metabolomic analysis of AC and AA, because of both AC and AA profile might reflect the mechanisms underlying development of metabolic syndrome. For instance, (i) plasma levels of AC with long saturated and monounsaturated even side-chain (C12-C18) could correlate with the activity of beta-oxidation in the tissues, which is frequently compromised in metabolic syndrome patients; and (ii) impaired metabolism of branched-chain AA was linked to insulin resistance, one of the key mechanisms involved in the development of the syndrome. To verify the potential of the usability of the plasma AC and AA profile as a complex biomarker in the area of metabolic syndrome, several model situation were explored using laboratory mice, namely: (i) early unmasking of different metabolic flexibility between obesity-prone and obesity-resistant mice (B6 vs A/J); (ii) development of obesity induced by high-fat diet (HFD) in B6 mice; and (iii) prevention of HFD-induced obesity by the combined intervention using *n*-3 PUFA and thiazolidinediones in adult B6 mice. Collectively, our results suggest that AC and AA plasma profile could mark and predict metabolic health.

## O-05 Lysophospholipids as biomarkers of trends to dyslipidaemia

A. Caimari[1], J.M. del Bas[1], M. Suárez[2], S. Suárez-García[2], F. Puiggròs[3], R.M. Valls[4], E. Llauradó[4], A. Pedret[4], R. Solà[4], L. Arola[2,3]

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Hamsters have been proposed as an interesting preclinical model for the study of pathologies related with lipoprotein metabolism due to the common features that these animals share with humans. Using this animal model, we aimed to identify early biomarkers of dyslipidaemia, a metabolic disturbance strongly related with a higher risk of cardiovascular disease. For this purpose, the plasma metabolomic profile of hamsters fed with a high fat diet (HFD) or treated with the hyperlipidemic chemical agent poloxamer 407 (P-407) during 4, 15 or 30 days was analysed by a non-targeted metabolomics approach (LC-MS/MS). Higher circulating levels of different lysophosphatidylcholines (lysoPCs) and lysophosphatidylethanolamines (lysoPEs) were found in hamsters fed the HFD at 4 and 15 days, when the dyslipidaemia was not yet completely developed. This metabolomic profile was partially maintained in the hamsters with a higher degree of dyslipidaemia (fed the HFD during 30 days or treated with P407), which also showed higher levels of some lysophosphatidylinositols (lysoPIs). To validate these results in humans, the circulating levels of a subset of lysoPCs, lysoPEs and lysoPIs were evaluated in 90 subjects distributed into 3 groups (n=30, 15 men and 15 women) according to their LDL-cholesterol levels: LDL<100 (optimal group, OP), LDL 100-129 (near optimal group, NO) an LDL 130-189 (borderline-high group, BH). Interestingly, both NO and BH groups showed lower circulating levels of lysoPEs 18:2 and 20:5 than OP subjects. Furthermore, NO individuals also showed lower levels of lysoPEs 18:1 and 20:4 compared to OP group, whereas the BH subjects presented increased levels of lysoPCs 18:0 and 20:0 as well as higher levels of lysoPIs 18:1 than the control group. In conclusion, our results suggest that low circulating levels of lysoPEs could be early biomarkers of dyslipidaemia whereas increased levels of lysoPCs and lysoPIs would indicate a higher degree of this pathology.

Acknowledgements: The research leading to these results received funding from ACC1Ó (TECRD12-1-0005) and from the European Union's Seventh Framework Programme FP7 2007-2013 under grant agreement nº 244995 (BIOCLAIMS Project).

## O-06 Blood cell transcriptomic-based early biomarkers of adverse programming effects on obesity and related risk factors

C. Picó, A. Palou

*Laboratory of Molecular Biology, Nutrition and Biotechnology (Nutrigenomics), University of the Balearic Islands and CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Palma de Mallorca, Spain.*

The challenge of preventing major chronic diseases requires reliable, early biomarkers. They could serve as tool to improve the effectiveness of its prevention, as well as to monitor the efficacy of nutritional and/or pharmacological interventions. Peripheral blood mononuclear cells (PBMCs) provide an attractive source of biomarkers, as they can be easily and repeatedly accessed in humans. Animal models with different predisposition to subsequent diseases, which may be obtained by interventions during the perinatal period, may be suitable to identify early biomarkers. In this sense, mild gestational calorie restriction in rats has been shown to program the offspring to develop later obesity and related metabolic alterations. In turn, studies in our laboratory have shown that the oral intake of appropriate amounts of leptin during lactation could be a strategy for reversing the effects of metabolic disorders induced as a consequence of developmental programming. We used these animal models to identify, in peripheral blood mononuclear cells (PBMCs), transcriptomic-based early biomarkers of programmed susceptibility to later disorders, and explored their response to neonatal leptin intake. Microarray analysis performed in PBMCs from the offspring of control and 20% gestational calorie-restricted dams (CR) allowed the identification of 224 known genes differently expressed between both groups of animals. Notably, leptin oral supplementation normalised 218 of the 224 mRNA levels identified in PBMCs associated to undernutrition during pregnancy. These findings support the importance of oral intake of leptin during lactation, a specific compound of maternal milk, which might be of relevance when considering strategies to treat and/or prevent the programmed trend to diseases acquired by inadequate foetal nutrition, particularly in susceptible subgroups. Moreover, this type of markers, once validated in sufficient studies in humans, may allow the identification and subsequent monitoring of individuals at early ages who are at greater risk of developing obesity and other pathologies, and whose alterations can be reverted by the intake of adequate amounts of leptin during lactation.

Acknowledgements: The research leading to these results has been funded in part by the European Union's Seventh Framework Programme FP7 2007-2013 under grant agreement nº 244995 (BIOCLAIMS Project), and by the Spanish Government (AGL2012-33692), and the Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición, CIBEROBN.

## O-07 The key biomarkers of inflammation

P.C. Calder

*Faculty of Medicine, University of Southampton, Southampton SO16 6YD and NIHR Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust and University of Southampton, Southampton General Hospital, Southampton SO16 6YD.*

Inflammation is a characteristic feature of many conditions and diseases. Irrespective of the trigger and the locus of activity, inflammation typically involves a common panel of cells and a common set of chemical mediators. There is a need for guidance on the assessment and interpretation of inflammatory biomarkers in nutrition studies. However, no consensus exists as to which markers best represent the various domains of inflammation which include acute, chronic and low-grade inflammation. Two recent pan-European expert groups have considered biomarkers of inflammation [1,2] and another one focussed on chronic low-grade inflammation in the context of overweight and obesity [3]. There are a number of modifying factors that affect the concentration of an inflammatory marker at a given time. Measuring the concentration of inflammatory markers in the bloodstream under basal conditions is probably less informative compared with data related to the concentration change in response to a challenge. A number of inflammatory challenges have been described. However, many of these challenges are poorly standardised. Patterns and clusters may be important as robust biomarkers of inflammation. Therefore, it is likely that a combination of multiple inflammatory markers and integrated readouts based upon kinetic analysis following defined challenges will be the most informative biomarker of inflammation. In BIOCLAIMS we have investigated the response of a range of inflammatory markers to a high fat meal challenge in normal weight and obese subjects. The kinetic evaluation was conducted over 6 hours following the meal. We further investigated the effect of including marine omega-3 fatty acids with the challenge meal or for a period of 12 weeks prior to the meal challenge. Key findings will be presented.

## O-08 Emergence of dicarbonyl stress as a key mediator of metabolic, vascular and renal health decline and related biomarkers revealed in BIOCLAIMS. New findings on Nrf2 regulation

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Dicarbonyl stress is the abnormal accumulation of dicarbonyl metabolites, particularly methylglyoxal (MG), leading to increased protein and DNA modification contributing to cell and tissue dysfunction in ageing and health decline. Enzymes metabolising dicarbonyls, particularly glyoxalase 1 (Glo1), provide an efficient and stress-response defence against dicarbonyl stress. Dicarbonyl stress is produced by increased formation and/or decreased metabolism of dicarbonyl metabolites, and by exposure to exogenous dicarbonyls. Genetic and metabolic factors link dicarbonyl stress to obesity and insulin resistance. In BIOCLAIMS studies we found evidence of increased MG in white adipose tissue and liver of mice on an obesogenic diet and prevention of this in obesity-resistant aP2-*Ucp1* mice. In clinical translation we found increased MG-modified protein adducts in obesity compared to non-obese subjects on an isocaloric diet. This was corrected by a low-calorie diet. MG-modified proteins were identified in skeletal muscle of ageing mice and decreased in mice with ectopic expression of *Ucp1* with a healthy ageing phenotype. Decreased expression of Glo1 was recently linked to cardiovascular disease in a large clinical cohort study. We have previously shown in experimental and clinical studies that dicarbonyl stress is a mediator of early decline in renal health.

A likely effective and safe strategy to prevent dicarbonyl stress is induction of Glo1 expression. In 2010 we discovered a functional ARE in human *Glo1* and induction of Glo1 expression by dietary bioactive activators of transcription factor Nrf2. Our prototype Glo1 inducer is in clinical trial in obesity for improved metabolic and vascular health. In BIOCLAIMS we discovered that Nrf2 works like a wireless sensor by continually moving in and out of the cell nucleus, in coordinated functional cycles of sensing, responding, inactivating and resetting in surveillance and initiating cyto-protective response to challenges to homeostasis. The "Nrf2 sensor" model provides a rational basis for improved design of dietary activators of Nrf2 and healthier foods.

Acknowledgements: We thank the Biosciences and Biotechnology Research Council (BBSRC), U.K. for research funding - Diet and Health Research Industry Club (DRINC) project grant no 07/84, and the European Union Framework Programme-7 for support for the BIOCLAIMS research programme, grant agreement no. 244995 and grant K/ZDS/002442.

## O-09 The BIOCLAIMS cohort as a resource for biomarker discovery and validation

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The *BIOCLAIMS cohort,* a total of 1310 study subjects*,* has been investigated by a common team of researchers of two partners of the BIOCLAIMS project, the Karl-Franzens University and Medical University of Graz; Austria. The cohort consists of 606 men and 704 women, covering the age range of 18-85 years (mean age 51.3±16.1 years). Apparently healthy subjects and patients attending the outpatient clinic of the Clinical Division of Nephrology of the Department of Internal Medicine of the Medical University of Graz were recruited after informed consent had been obtained. The Study protocol was approved by the Ethics Committees of the Medical University and Karl-Franzens University of Graz.Recruitment was performed according to a pre-defined inclusion/exclusion criteria. Blood was drawn after an overnight fast and additional biomarker sources such as spot urine and 24h urine samples, buccal mucosal cells, hair and nails were collected. Standardized pre-analytical sample work-up ensured best possible quality of the samples which have been stored at -80°C until analysis during the project duration and beyond (*BIOCLAIMS sample collection*). All study subjects underwent comprehensive dietary (240-item food-frequency questionnaires, prospective 5-day food records), anthropometric (weight, height, body mass index, skinfolds, subcutaneous adipose tissue distribution at 15 sites at the left and right side of the body [Lipometer®], bioelectrical impedance), clinical (blood pressure, intima-media thickness (IMT) of A. carotis, flow-mediated dilatation of A. brachialis), and biochemical characterization, resulting in over 500 variables, including pro-inflammatory genotypes, collected from each subject to date.

Subsets of the volunteers participated also in follow-up studies. These studies are focused on within-subject variability of the established and new biomarkers under investigation:

- The *Menstrual Cycle Study* included 4 investigations of 28 women with a natural menstrual cycle at time points in the early and late follicular and early and late luteal phase determined on the basis of basal temperature measurements.
- The *Seasonal Variability Study* included 12 investigations of 52 study subjects (50% men and 50% women) at monthly intervals of an entire year.
- The *Day-to-Day Variability Study* included 5 investigations Monday through Friday of 12 study subjects (men and women) at the same time of the day.

Samples of 4 subsets of the study cohort have been further used in the *BIOCLAIMS Integration (BIG) Study* to *validate new biomarkers* in different partner laboratories. These included study subjects with mildly impaired renal function (glomerular filtration rate 30-60 ml/min/1.73 $m^2$), mildly impaired vascular function (IMT left and right >75$^{th}$ percentile of age and gender-specific reference values) and mildly impaired glucose metabolism (HOMA index 2.5 plus HbA1c 38.8-44). These groups were compared with a group of so-called "super healthy" subjects. These study subjects were identified as having all relevant clinical chemistry routine variables within the normal range with a 10% tolerance range, absence of IMT left and right >75$^{th}$, HOMA index <2.5 and HbA1c <38.8, and not taking any medications. Given their comprehensive characterization, these subjects could serve as *human super-healthy model* in the substantiation of biomarkers of health.

Acknowledgements: *Funded by the European Union's 7$^{th}$ Framework Programme FP7 2007-2013 under grant agreement nº 244995 (BIOCLAIMS Project) and the Federal Ministry of Science, Research and Economy of Austria*

## O-10 Selected nutrient responsive biomarkers of metabolic syndrome.

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Obesity and prediabetes is associated with low grade inflammation due to the gluco- and lipotoxicity resulting in development of diabetes type 2 and cardiovascular complications. Postprandial period (which can be standarized by OGTT or OLTT) caused transient aggravation of biochemical markers of inflammation accompanied by transient changes in TG, LDL density, lipids, lipid droplet content (genes, miRNA enzymes), cytokines - modulators of gene expression, adipose tissue differentiation, insulin secretion and sensitivity. Glucose-dependent insulinotropic polypeptide (GIP) increases postprandially, however lipid overload (OGTT) causes higher, longer lasting effect in comparison to OGTT. Carboxylated osteocalcin (Gla-OC) interacts with $Ca^{2+}$ and participates in bone remodeling, and tissue calcium level, whereas the undercarboxylated form (Glu-OC) has a hormone-like function in energy metabolism. In obese patients serum levels of Gla-OC were significantly lower and inversely correlated with BMI, hsCRP, visfatin concentration. Conversely, Glu-OC inversely correlated with fasting insulin level and HOMA index. In comparison to healthy obese volunteers, prediabetic subjects presented lower Glu-OC levels. Three months of caloric restriction or DHA/EPA supplementation exerted beneficial effects expressed by a reduction of insulin, resistance markers, lowering of blood triglycerides, normalization of osteocalcin and the decrease of plasma GIP levels. The Glu/Gla osteocalcin and GIP may be early candidates of diet-regulated markers of lipotoxicity –induced inflammation resulting in insulin resistance. Dietary supplementation with n-3 PUFA affects the fatty acid serum profile (saturated, mono- and n-3/n6 PUFA), modifying low-grade inflammation by increasing of lipoxins, resolvins and similar acting autacoids.

Acknowledgements: Supported by EU FP7 BIOCLAMS, Grant agreement no. 244995, NCN grant no. K/PBN/000001;and grant K/ZDS/004497.

## O-11 Knowledge and data driven development of nutrigenomics-based biomarkers of robustness

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The BIOCLAIMS project aims to identify and validate nutrigenomic-based biomarkers for assessing the potential effectiveness and benefits of health-promoting food compounds. A nutrigenomic-based "biomarker of robustness" is defined as a marker for which an integrated analysis of its gene/protein and/or metabolite levels as well as available biological knowledge has provided sufficient evidence to reflect the health status of a physiologically relevant process.

In the BIOCLAIMS project an extensive set of human, animal and cross-sectional studies were performed in which many potential nutrigenomics-based biomarkers were measured. Our objective was to allow for validation of biomarkers across studies and organisms with the aim to enhance scientific evidence for effects of dietary intervention on identified biomarkers, by storing all data and results in a standardized way in the BIOCLAIMS database.

Network analysis enables integration and visualization of multi-layered experimental datasets and prior knowledge. All BIOCLAIMS studies focusing on n-3 fatty acid effects were collected to deliver a Proof of Concept for selection of nutrigenomic-based "biomarker of robustness".

The prior knowledge was captured in a knowledge database, which was made available on a website called the 'BIOCLAIMS Data Integration Website'. For the Proof of Concept analysis, data from 2 human intervention studies, 1 cross-sectional study and 1 mouse study were available in the BIOCLAIMS database. The selected data integration approach allowed us to identify a set of nutrigenomics-based "biomarkers of robustness" based on presence of different levels of scientific evidence such as analytical robustness, association with disease, physiological connection to health endpoint, and mechanistic evidence.

The BIOCLAIMS data Integration Website, the BIOCLAIMS database and our data integration pipeline offers a sustainable knowledge resource and toolbox for assessment of health effects of nutrient interventions and support of health claims made on food products.

Acknowledgements: Jildau Bouwman, Marijana Radonjic, Thomas Kelder.

## O-12 Authorised Claims in the EU: Status, claims on hold, harmonisation and next steps

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Nutrition and health claims are regulated in the EU by Regulation (EC) No 1924/2006 of 20 December 2006. The Regulation sets the definitions, general principles and conditions as well as the procedures for the adoption of the implementing measures that authorise or reject claims.

Only authorised claims can be used in the labelling, advertising and presentation of foods in the EU. One of the most important criterion for their authorisation is that they should be substantiated by generally accepted scientific evidence of the highest possible standard. Such evidence is substantiated by the European Food Safety Authority (EFSA).

Over 2200 claims have been evaluated until today. Of those 260 have been authorised while just over 2000 have been rejected. About 2000 claims, mainly concerning botanical substances, are still under consideration ("on hold") and until a final decision on them they can remain on the market, provided the other provisions of the Regulation are fulfilled.

## O-13 Labelling, advertising and presentation of foods. Compliance and infringement

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The presentation of foods, including health and nutrition claims, has been harmonised under Europe Law. Therefore the rules for compliance, the obligations imposed upon stakeholders, are relatively well defined and uniform. However this harmonisation does not cover penalties nor infringement procedures, which are still subject of national law, with the exception of the system for food alerts, which, in itself can represent the harshest of penalties.

The main principles ruling consumer information are that companies are responsible for the products they manufacture or distribute and that they must not mislead consumers when presenting their foods products. If the presentation of the product involves a nutrition or health claim, stricter rules apply since only those claims specifically authorised can be used to promote a food product. Furthermore, there is an absolute limit to health and nutrition claims, namely that they must not declare or give the impression that a food has medicinal effects. However in practical terms, in a single market it is not always easy to identify who is ultimately responsible for a product, nor to define the boundary between commercial freedom of speech and the misleading of consumers, or whether a claim respects both the text and intention of the approved claim, or whether or not the health effects are medicinal.

Infringement is a matter of national law. Procedures vary from country to country and the perception of national authorities on the nature of the infringement and its consequences can differ. Furthermore, the use of the Rapid Alert System can be the most serious consequence of an infringement, the real penalty being not the sanction itself but the communication of a problem to the public, the damage to reputation. This system, useful as it is as a tool to protect public health and other consumer interests, poses deep procedural and defence related problems.

A complex legal panorama in a changing society.

## O-14 Nutrition of mothers and young children in the first 1000 days: Learning's from human milk and what to apply?

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The first 1000 days of life, starting from conception are a period of very rapid growth and development. Nutritional requirements in this period are special. The development of the gastro-intestinal (GI) tract during early life is crucial for tuning the infant's metabolism and immune responsiveness towards requirements. The colonization of the infant's intestines by microbial communities is an aspect in this tuning process that, like so many others, is influenced by maternal milk compounds.

WHO 2011 recommends exclusive breastfeeding (BF) for first six months, and continued breastfeeding up to two years and beyond. After exclusive breastfeeding, adequate complementary foods are recommended.

Breastfeeding has numerous positive effects, both short- and long term, on the newborn and benefits for the mothers that are measurable on a public health scale. In addition to macronutrients, which provide energy and building blocks for tissue growth, human milk (HM) contains a large number of compounds that modulate functional aspects of metabolism. Mature HM contains low amounts of protein 13 g/L. The ratio of caseins and whey proteins is reflecting functional and nutritional needs and deviations from the natural ratio induce changes in postprandial responses. Research on protein requirements and breast milk composition over the last decades has been translated into specific compositional recommendations for dietetic products for infant nutrition. Depending on the diet and genotypes, the lipid fraction of HM contains a variable ratio of n-3 and n-6 long-chain polyunsaturated fatty acids (PUFAs) driving regulation of energy homeostasis and cognitive development. The importance of DHA in particular to brain development has been acknowledged by EFSA. HM contains 10-12 g/l of human milk oligosaccharides (HMOS) with complex molecular structures affecting the intestinal microbiota and the developing immune system. The prebiotic effect of HMOS could be translated into a concept for prebiotic nutrition for the first 1000 days of life by applying specific mixtures of non-HMOS prebiotics. With this concept, a Bifidus-dominated microbiota, a reduction of pathogens, and stool characteristic ssimilar to breastfed infants can be achieved (similar pH, pattern of short chain fatty acids, stool frequency, and stool consistency). Other proven positive effects of prebiotics in infant nutrition are an improved maturation of the GI tract and reduced incidences of infections and allergic symptoms. The prebiotic effect is an established scientific fact. More recently, it was discovered that HM contains beneficial bacteria. Their relevance and physiological effect for mother and child is currently being explored.

Future studies on the causes and consequences of the variation in HM composition related to maternal nutrition, health and lifestyle will deepen our understanding of the nutritional needs of mothers and their young children during the first 1000 days.

## O-15 Health claims which might arise in relation to riboflavin use in the lowering of high blood pressure: a novel gene-nutrient interaction

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Hypertension, defined as a systolic/diastolic blood pressure of 140/90 mmHg or greater, is estimated to carry a 3-fold increased risk of developing cardiovascular disease (CVD). Evidence from genome-wide association studies has identified an association between blood pressure and the gene encoding the folate-metabolising enzyme, methylenetetrahydrofolate reductase (MTHFR). Recent meta-analyses of observational studies show an increased risk of hypertension in people homozygous for the 677C→T polymorphism in MTHFR. Riboflavin in the form of FAD acts as a cofactor for MTHFR and the variant enzyme is known from molecular studies to become inactive as a result of having an increased propensity to dissociate from FAD. We have shown that CVD patients with the relevant MTHFR 677TT genotype (compared to CC or CT genotypes) have significantly higher blood pressure, and that blood pressure was highly responsive to riboflavin intervention, specifically in the TT genotype group. Further investigations confirmed this gene-nutrient interaction in hypertensive patients (with and without overt CVD), and showed that the blood pressure lowering effect of riboflavin in the TT genotype group was independent of the number and type of antihypertensive drugs taken. Although the precise mechanism linking this polymorphism to hypertension remains to be established, it would appear that the biological perturbation which leads to higher blood pressure in individuals with the MTHFR 677TT genotype is modifiable by correcting the variant MTHFR enzyme through enhancing riboflavin status. Issues with respect to possible Article 13.5 and 14 Claims arising from such a gene-nutrient interaction will be highlighted.

## O-16 Health claims and probiotics: what and how?

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Currently, legislation regulates the use of health claims for probiotics in most parts of the world, although differences in criteria have created country-specific scenarios. Within the European regulatory framework, the approval of a health claim application requires data of the identity of the probiotic microorganism at the species and strain level, the definition of the health benefit, which should be specific and measurable, and the substantiation of a cause-effect relationship for which controlled human intervention trials are of primary importance. These criteria do not differ substantially from those established in the first guidelines for the evaluation of probiotics in foods published by an Expert Consultation group, working under the umbrella of Food and Agriculture Organization (FAO) and World Health Organization (WHO) in 2002. Nevertheless, at that time these criteria were not legally enforceable and, therefore, the standards proposed were only partially followed by the different actors in the field. Intervention studies with probiotics have not always focused on endpoints considered to reflect *per se* beneficial physiological effects, while substantial effort has been made to assess secondary endpoints (e.g. lactobacilli or bifidobacteria numbers in stools) less relevant to substantiate claims. The EU Regulation has also introduced new challenges into the scene, including the need to substantiate claims for a general healthy population, while most intervention studies have been conducted in diseased populations, and the identification and validation of biomarkers serving as predictors (risk factors) of disease in humans. Indeed, after eight years of evaluating health claims in the EU, there is only one health claim approved related to live yogurt cultures and their ability to improve lactose digestion. Similarly, in the US no health claims on probiotics have been approved by the FDA yet; however, structure-function claims can be made without such approval, facilitating the communication of probiotic effects backed by less robust scientific evidence. In the EU, to improve the understanding of the scientific criteria applied for evaluating health claims related to gut and immune function, and to better define how a probiotic claim could be substantiated, the EFSA guidance document published in 2011 has been recently updated and will be discussed in the light of scientific progress and the global regulatory context.

Acknowledgements: grant nº 613979 (MyNewGut) from the Seventh Framework Programme of the European Union.

## O-17 Strategies and challenges in fat related claims. Driving innovation and value based on health and sustainability

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There are several health claims on fats approved by EFSA, mainly related to LDL-cholesterol lowering and CVD endpoints. These claims are full in line with the scientific consensus and major dietary guidelines.

Unilever innovations are based on this consensus and so is the Unilever Sustainable Living Plan (USLP), which sets strict targets and achievements both on health and nutrition (saturated, insaturated fats and salt) and on reducing our environmental impact (sourcing, carbon emission).

Our main challenges are the contradictory voices in the scientific world, which show diverse interpretation of the same results or challenge the validity of substantiated claims. Some contradictory voices have been recently spread by media, taking consumers to confusion and challenging the scientific consensus. New science should always be put in perspective and take into account global evidence to spread consistent messages and ultimately protect public health.

## O-18 HEALTH CLAIMS: Collaborative Industrial Research

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Regulation on Nutrition and Health Claims made on foods of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods, changed the scenario for Food Companies, and mainly for Small and Medium Enterprises (SMEs). In Spain, this new scenario have given the opportunity to some Industrial Stakeholders of Spanish Food Industry as Laboratorios Ordesa to collaborate together in several Industrial consortiums, in order to investigate and develop new functional ingredients and products with the objective to substantiate new Health Claims or improve the scientific evidence of these ingredients or products. All these Industrial Collaborative Research Projects have been funding by Centre for Industrial Technological Development (CDTI) and had involved 29 companies.

We can stand out some examples of this kind of projects:

SENIFOOD PROJECT (Functional ingredients and development of diets for elderly population), funding by CDTI's CENIT programme (large scale and major scientific-technical consortium projects aimed at planned research in future technology areas and with potential international projection, whose mission is ot generate new knowledge that may be useful to create new products, processes or services or to integrate strategic technologies)

INCOMES PROJECT (Guidelines for substantiate health claims in foods related with immune, cognitive and Metabolic Syndrome) funding by CDTI's INNPRONTA programme (large integrated industrial research projects, of a large-scale and strategic nature, which serve to develop new technologies in forward-looking technological areas with economic and commercial prospects at the international level.)

SMARTFOOD PROJECT (Industrial Research and Development of SMARTFOODS) funding by CDTI's CIEN programme.

## O-19 Smart foods and targeted food supplements

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Recent advances in the Nutrition field, mainly associated with the knowledge generated by Nutrigenomics and its area of influence, are contributing to support the development of novel food products. Current scientific background is enabling the design of innovative food products, targeted for specific groups of people, or even more, intended for individual genotypes. Proper and individualized nutrition is envisaged to promote healthier citizens. Therefore, the aim beyond this novel approach is to provide with healthier eating options to consumers.

Alimentomica is a technology based company, spin off of UIB (number 001), aiming the development of novel food products and services in the field of functional foods. Main strength of the company is the long and outstanding scientific career of the founding partners in the field of molecular nutrition and obesity. The portfolio of Alimentomica involves the development of food products intended to optimize early nutrition in children as well as in adults, particularly focused on the prevention of obesity. The development and launching of Metigentity, a nutrigenetic test based on genetic variants potentially related to obesity, allows for personalised nutrigenetic counselling. These two areas of activity are now converging on the development of a new project for the achievement of smart foods intended for specific nutritional purposes and/or bearing health claims. The cornerstones of smart foods and targeted food supplements are the identification of bioactive compounds and their impact on functional biomarkers, the supportive scientific evidence for their beneficial role together with evidences of specific nutrient requirements associated to target populations, as considered also from a classical approach (i.e. women, old, sports people, etc) and/or integrating their genetic blueprint.

## O-20 New strategies on HEALTH CLAIMS – Replacing foods components as basis for HEALTH CLAIMS

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Until recently, health claims were only considered when the health effect was directly linked to the "action" of a component. There were based on the DIRECT health effects of one or several components present in a food (intentionally added or naturally present), as for example claims linked to phytosterols added to foods or vitamins and minerals present in foods.

There are now new strategies, new approaches developed in a different way. The health effect is due to the absence, the presence or the replacement of a component IN THE DIET. Which is obtained by the reduction, the withdrawal or the replacement of the component in the specified FOOD, part of the diet.

To illustrate this new approach, we will take 2 examples :

- Article 14 For fats and oils, The replacement of saturated fats with unsaturated fats in the diet which has been shown to lower/ reduce blood cholesterol. This claim is allowed by the COMMISSION REGULATION (EU) No 1226/2014 of the 17$^{th}$ of November 2014
- Article 13, still pending, for dairy products. Reduction of the content of lactose in the dairy product and thus, the lactose content of the diet. "Consumption of lactose in amounts exceeding individual tolerances may lead to the occurrence of symptoms of lactose intolerance in lactose intolerant individuals; consumption of foods with reduced amounts of lactose may help to decrease gastro-intestinal discomfort caused by lactose intake in lactose intolerant individuals."

This "new angle" for health claims reopens the debate on health claims related to foods WITHIN A DIET, and thus expand the area of the possibility to claim on foods.

## O-21 CIBER: a new consortium for human biomedical research studies in Spain. multicenter facilities

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CIBER is a Spanish public consortium, whose main purpose is the promotion and protection of health through the development of research, within the following areas of research: Bioengineering, Biomaterials and Nanomedicine (CIBERBBN); Rare Diseases (CIBERER); Respiratory Diseases (CIBERES); Hepatic and Digestive Diseases (CIBEREHD); Epidemiology and Public Health (CIBERESP); Mental Health (CIBERSAM); Diabetes and Associated Metabolic Disorders (CIBERDEM); Physiopathology of Obesity and Nutrition (CIBEROBN). Consortium created and funded by the Instituto de Salud Carlos III in order to promote and cooperate in scientific research, development of knowledge and transfer of it to the society. In that sense, a consortium is characterized by:

- It boosts and spreads technology development and its use
- The costs and expenses are shared and therefore more affordable
- It is easier to obtain resources, as the competences of the different entities are offered together.
- It is a driving force for new actions and joint policies.
- It acts as a learning platform.

In this sense, some selected shared technology platforms and facilities CIBER provides are: a Metabolomic platform (CIBERDEM); Advanced Imaging & Genome Analysis Services (CIBEREHD), e-CATCH (genetic counselling in the Colorectal Cancer - CIBEREHD), SEFALer (phenotyping of rare-diseases animal models - CIBERER), ORPHANET (Spanish node for the portal for rare diseases and orphan drugs - CIBERER); advanced imaging for multimodal diagnosis (CIBERBBN), Neuroimaging (CIBERSAM), therapeutic-targets-identification platform (CIBERSAM), among many others. Finally, there are specialised biobanks and bioinformatics services. Moreover, we offer support for clinical trials, communication, technology transfer, etc.

CIBER works as a the perfect umbrella for the joint development of R&D&I activities in 8 main areas of knowledge in Biomedicine; and provides an unique opportunity for researchers, companies, business & patients association, among other stakeholders, for collaborating with the best staff in our country and for having access to state-of-the-art facilities and know-how.

## O-22 AECOSAN as national EFSA focal point: an interface for scientific cooperation

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Scientific cooperation between the European Food Safety Authority (EFSA) and the Member States is an essential part of the founding Regulation of EFSA and, therefore, a priority in its Strategies for Cooperation and Networking. At national level, the Spanish Agency for Consumer Affairs, Food safety and Nutrition (AECOSAN) is responsible for interlocution with the European Food Authority being the Focal point of EFSA in Spain". This involves the double task of distributing the information from EFSA to the Spanish scientific community and stakeholders and of transmitting Spanish criteria and viewpoints on the different aspects related to food safety to the Authority. In addition AECOSAN invest many efforts in trying to improve and increase the role and weight of the Spanish scientific community within the Authority.

Overall scientific cooperation occurs through the national competent authorities, scientific organizations and individual experts. In coherence with the target the scientific cooperation is articulated with different tools as advisory boards, networks of experts, panels and scientific committee, and projects with financial support.

AECOSAN, as EFSA focal point, is the competent authority in Spain that deals with the different processes related with the applications of different *regulated products* assessed by the EFSA. AECOSAN is the national authority in charge of the process of submitting to EFSA health claims applications from the different stakeholders.

# POSTER ABSTRACTS

## P-01 Muscle mitohormesis promotes cellular survival via protein turnover and induced amino acid stress response

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Recent studies on mouse and human skeletal muscle demonstrated the important link between mitochondrial function and the cellular metabolic adaptation. To identify key compensatory molecular mechanisms in response to chronic mitochondrial distress, we made use of transgenic mice with ectopic skeletal muscle respiratory uncoupling (UCP1-TG) as model of muscle-specific compromised mitochondrial function. We performed a whole genome microarray analysis in skeletal muscle combined with qPCR, protein expression, plasma and muscle metabolomic analyses as well as functional studies.

Here, we describe a detailed metabolic reprogramming profile associated with mitochondrial perturbations in skeletal muscle, triggering an increased protein turnover and amino acid metabolism with induced biosynthetic pathways of serine, one-carbon and glycine (SOG), the longevity promoting polyamine spermidine as well as the transsulfuration pathway fluxes related to an increased glutathione metabolism. Strikingly, consistent muscle retrograde signaling profiles were observed in acute stress states such as muscle cell starvation and lipid overload, muscle regeneration and heart muscle inflammation, but not in response to exercise.

Conclusively, we provide evidence for a key compensatory stress-signaling network that preserves cellular function, oxidative stress tolerance and survival during conditions of increased muscle mitochondrial distress, a metabolic reprogramming profile so far only demonstrated for cancer cells and heart muscle. This enlarges the emerging concept of muscle mitohormesis and opens up therapeutic opportunities for many metabolic diseases and possibly age related muscle decline.

Acknowledgements: Supported by European Union's Seventh Framework Program FP7 2007-2013 under grant agreement nº 244995 (BIOCLAIMS Project) and Leibniz Society (SAW-2013-FBN-3)

## P-02 Odd-chain fatty acids as a biomarker for dietary fiber consumption?

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Odd-chain fatty acids (OCFA: pentadecanoic acid (15:0) and heptadecanoic acid (17:0)) are inversely associated with type 2 diabetes [1]. We propose that OCFA reflect the intake of dietary fibers as intestinal fermentation of fibers mainly results in the short-chain fatty acids (SCFA) acetate (Ac), butyrate (Bu) and propionate (Pr). The latter is used for long-chain fatty acid synthesis leading to an increased proportion of odd-numbered fatty acids. Increasing colonic propionate has recently been shown to prevent weight gain and deterioration in insulin sensitivity in overweight humans [2].

To corroborate our hypothesis, mice were fed semi-synthetic high fat diets supplemented either with 10% dietary fibers (non-fermentable cellulose (HFC), fermentable inulin (HFI)) or different Ac:Pr ratios: high acetate (HAc; 2.5:1 Ac:Pr) and high propionate ratio (HPr; 1:2.5 Ac:Pr), respectively. To support the in vivo data HepG2 cells were incubated with different Ac:Pr ratios for 48h.

Inulin supplementation resulted in higher total SCFA-production and increased propionate concentrations in cecum and portal vein plasma compared to HFC. Dietary inulin- and SCFA-supplementation (independent of the SCFA type) reduced hepatic lipogenesis and altered long-chain fatty acid composition in liver and plasma phospholipids. Especially, the formation of OCFA was enhanced when propionate concentrations were increased either by dietary propionate supplementation or higher propionate production from inulin. This was confirmed by cell culture experiments, showing a strong correlation between propionate supplementation and the production of OCFA in hepatic cells.

Our data indicate that a high intake of fermentable dietary fibers is associated with an increased formation of propionate, which in turn results in an enhanced OCFA production. Therefore, OCFA could be used as a biomarker for the intake of fermentable dietary fibers linked to colonic propionate production.

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Acknowledgements: Supported by the German Research Foundation (DFG, grant # KL613/18-1).

## P-03 Oxygen restriction as challenge test reveals early diet-induced health effects

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Challenge tests stress homeostasis and may reveal deviations in health that remain masked under unchallenged conditions. Ideally, challenge tests are non-invasive and applicable in an early phase of a study. The response to oxygen restriction (OxR; mild normobaric 12% $O_2$) measures the flexibility to adapt metabolism. Metabolic inflexibility is one of the hallmarks of the metabolic syndrome and underlying insulin resistance. We used male C57BL/6JOlaHsd adult mice fed a low-fat (LF) or high-fat (HF) diet for only five days. Indirect calorimetry was used to assess the response to OxR. Serum markers, including protein glycation/oxidation, and gene expression in liver and white adipose tissue (WAT) were analyzed.

Although HF-fed mice had a significant higher body weight (BW) after five days of feeding, HF-fed and LF-fed mice did not differ in calorimetric values under normal conditions nor in fasting state. Moreover, the subgroups of mice that were fasted and exposed to OxR during the last 6h showed no differences in BW (gain), nor in blood glucose levels. Exposure to OxR, however, revealed significant differences in substrate use with the HF-fed mice showing higher fatty acid oxidation levels and oxygen consumption, while their activity was similar to LF-fed mice. Furthermore, hepatic and WAT transcript levels differed significantly between both groups indicating differences in their adaptation to OxR. Only HF-fed mice showed increased hepatic lactate/glucose metabolism upon OxR, while LF-fed mice showed an increased oxidative stress response in WAT. The adaptation in HF mice appeared to be dampened, associated with increased serum markers of protein glycation/oxidation, whereas these changes were absent in LF mice.

In conclusion, an oxygen restriction challenge test is a promising new method to test food products on potential beneficial effects for metabolic health.

Acknowledgements: Supported by EU FP7 BIOCLAIMS Project, grant 244995.

## P-04 The BIOCLAIMS study at the University of Southampton: The effect of obesity on post-prandial incorporation of marine omega-3 fatty acids into plasma triacylglycerol

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Most studies have focused on normal weight subjects when investigating omega-3 fatty acid incorporation into plasma post-prandially [1]. There is very limited information in obese subjects.

This study investigated whether obese subjects show a different pattern of incorporation/clearance of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the plasma triacylglycerol (TAG) pool during the post-prandial period compared to normal weight subjects.

For full details of the study design, please refer to abstract by Childs CE *et al*. 100 healthy adults (50 normal weight (BMI of 18.5-25 $kg/m^2$) and 50 obese (BMI of 30-40 $kg/m^2$)) were recruited into a double blinded, randomised, cross over, post-prandial trial. The subjects attended 3 high fat dietary challenge days. Between visit 2 and 3 (12 weeks duration) volunteers were randomized to take fish oil or placebo capsules daily. At each visit fasted baseline and serial blood samples were collected until 6 hours post meal + capsules (placebo or fish oil). Fatty acid compositional analysis was conducted on the plasma TAG using gas chromatography.

Total circulating TAG levels were higher and showed reduced clearance in the obese group (P=0.002). Plasma TAG EPA incorporation was comparable between normal weight and obese subjects consuming fish oil with their meal. Obese subjects showed greater incorporation and retention of DHA in plasma TAG (P=0.004). Following the 12 week supplementation with fish oil, obese subjects released more EPA and DHA into their post-prandial TAG pool than lean subjects ($P<0.05$).

Obese subjects have reduced TAG clearance after consuming a high fat meal. This results in DHA being retained in the circulating TAG pool for longer than in normal weight subjects. After a period of chronic fish oil supplementation obese subjects are able to make EPA and DHA available to newly synthesised TAG during the post-prandial period. It is not clear what the mechanism for this is.

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## P-05 Carboxylated and undercarboxylated osteocalcin as the marker of metabolic complications of human obesity and prediabetes

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Carboxylated osteocalcin (Gla-OC) participates in bone remodeling, whereas the undercarboxylated form (Glu-OC) takes part in energy metabolism. This study was undertaken to compare the blood level of Glu-OC and Gla–OC in non-obese, healthy obese as well as prediabetic volunteers and correlate it with the insulin resistance and inflammation markers.

Non-obese (BMI < 30kg/m$^2$, n=34) and obese subjects (>30 BMI<40 kg/m$^2$, n-98), aged 25-65 yrs were included in the study. The non-obese control, healthy obese and obese with biochemical markers of prediabetes patients, as well as the subgroups with obesity and low or high Gla-OC or Glu-OC were considered for analysis. Venous blood was sampled for determination of Gla-OC, Glu-OC, lipid profile, selected parameters of inflammation (hsCRP, IL-6, sE-selectin, sPECAM-1, MCP-1) and adipokines: leptin, adiponectin, visfatin and resistin. Blood was collected during oral glucose tolerance test (2h OGTT) and oral lipid tolerance test (8h OLTT) for measurement of glucose, insulin, FFA, TG and glucose-dependent insulinotropic polypeptide (GIP). Insulin resistance was estimated by HOMA-IR index and using calculated an oral glucose insulin sensitivity index. In the whole group of obese patients the level of Gla-OC was lower comparing to non-obese subjects. The serum level of Gla-OC inversely correlated with hsCRP, visfatin, BMI. In turn Glu-OC inversely correlated with fasting insulin level and HOMA IR index. In comparison to healthy obese volunteers, prediabetic subjects presented reduced Glu-OC level. Blood glucose, insulin and GIP level as well as HOMA-IR index were higher in this group pointing to decreased insulin sensitivity.

Our results argue for the suggestion, that in obesity, decreased blood concentration of Glu-OC may be early marker of insulin resistance, whereas the lower Gla-OC level could be associated with the appearance of early symptomes of inflammation.

Acknowledgements: Supported by EU FP7 BIOCLAIMS, Grant agreement no. 244995 grant K/ZDS/004498 and NCN grant no. K/PBN/000001.

## P-06 GIP and lipids changes in obese patients supplemented with n-3 PUFA during caloric restriction

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Higher plasma GIP level may reflect compensatory mechanism to overcome the diminished islet response in obese patients. The study was aimed to analyze GIP and lipids response to 3-month intervention in terms of n-3 PUFA supplementation along with caloric restriction.

A total of 62 obese patients without any metabolic disorders were included into the study. Patients were assigned to low calorie diet (1200-1500 kcal/day) and were supplemented either with n-3 PUFA (DHA:EPA; 5:1) 3x600mg daily, or placebo (corn-oil), for 3 months. Effects of the intervention on plasma fatty acids profile was assessed by LC-MS/MS.

The detail analysis of plasma fatty acids was performed in relation to GIP status. Supplementation with n-3 PUFA significantly decreased total content of plasma PC FA only in patients with higher GIP level. Caloric restriction alone decreased the saturated/unsaturated FA ratio in Low GIP in contrast to High GIP group. In the High GIP group n-3 PUFA supplementation resulted in decrease of saturated fatty acids: palmitic (16:0), stearic (18:0), miristic (14:0) as well as mono-unsaturated FAs: palmitooleinic (16:1), oleic (18:1) but not PUFA. The n-3 PUFA supplementation resulted in reduction of plasma n-6 PUFA: linoic (18:2) and arachidonic (20:4), which was also pronounced within High GIP group.

We conclude that supplementation of the low-calorie diet with n-3 PUFA significantly improves plasma lipid profile in obese patients characterized by high GIP plasma level. The GIP plasma level may indicate subjects who would potentially benefit from n-3 PUFA supplementation.

Acknowledgements: We are grateful Morten Bryhn MD, PhD and EPAX AS Norway for kind support of JUMC BIOCLAIMS study with the EPAX 1050TG and placebo capsules. Study supported by EU FP7 BIOCLAMS, Grant agreement no. 244995, NCN grant no. K/PBN/000001 and grant K/ZDS/004497.

## P-07 Anti-inflammatory effects of transcellular EPA and DHA metabolites in prediabetes

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It was postulated that ω-3 polyunsaturated fatty acid (PUFA): EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) have inhibitory effects on low-grade inflammation associated with obesity and prediabetes. The ω-3 PUFAs inhibit synthesis of pro-inflammatory arachidonic acid derivatives, the expression of inflammatory cytokines and endothelial adhesion proteins. Relationship between therapeutic response and modifications of transcriptome expression in obesity or metabolic syndrome remains open for further exploration.The aim was analysis of the changes in gene expression of whole blood RNA and the associated changes in metabolic pathways following ω-3 PUFA supplementation.

Blood of women with obesity was sampled before and after three-month supplementation with low doses of ω-3 PUFA (3x600mg/day DHA:EPA) (5:1)). The erythrocyte ω-3 PUFA concentration and plasma lipoxins A4, A5, resolvins D1, D2, protectin X were measured (LC-MS/MS) and correlated with the inflammatory markers (sE-Selectin, s-VCAM-1, sPECAM-1, hsCRP, IL-6, MCP-1) were determined using ELISA kits. In parallel the significant changes of gene expression was estimated using microarray.

Obese women responded to ω-3 PUFA enriched diet with decrease of pro-inflammatory markers and increase of anti-inflammatory DHA-derived eicosanoids. The microarray data indicated ω-3 PUFA activation of the NRF2 and PPAR-alpha target genes related to beta-oxidation pathway, phospholipid synthesis, mitochondria electron transport chain proteins and antioxidant enzymes. The decrease of an expression inflammatory cytokines – (NFKB-target genes) was also found.

The ω-3 PUFA-derived eicosanoids may regulate early inflammation by PPAR-alpha activation, inhibition of NFKκ-controlled transcription of pro-inflammatory cytokines and by activation NRF2 signalling resulting in the up-regulation of antioxidant enzymes. Thus the low-grade inflammation in obesity can be inhibited the transcellular ω-3 PUFA metabolites.

Acknowledgements: We are grateful Ph dr Morten Bryhn and EPAX AS Norway for kind support of JUMC BIOCLAIMS study with the EPAX 1050TG and placebo capsules. Supported by EU FP7 BIOCLAMS, NCN grant K/PBN/000001.

## P-08 The BIOCLAIMS study at the University of Southampton: the effect of BMI on adipose tissue fatty acid composition and response to chronic marine omega-3 fatty acid supplementation

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The BIOCLAIMS Study at the University of Southampton assessed the acute and chronic effects of omega-3 fatty acid (n-3FA) supplementation on the post-prandial metabolic and inflammatory responses to a high-fat dietary challenge (see poster by Childs et al. for full details of the study design). Adipose tissue (AT) biopsies collected prior to the challenge at challenge days 1 and 3 are now being assessed as a secondary outcome to this study.

We investigated the influence of obesity on the fatty acid composition of AT and its response to chronic n-3FA supplementation. The proportion of different fatty acids in the total lipid extract of AT was measured by gas chromatography and statistically analysed to investigate the effect of BMI on baseline fatty acid levels and the effect of chronic n-3FA supplementation.

There were significant differences in the fatty acid composition of adipose tissue at baseline between lean and obese participants when adjusted for their age. Obesity was associated with higher proportions of 16:1n-7, 18.1n-7, 20:3n-6 and arachidonic acid (AA) ($P$ = 0.044, 0.000, 0.000 and 0.000 respectively), as well as with lower proportions of myristic acid, 18.0, α-linolenic acid, 20:1n-9 and 20:4n-3 ($P$ = 0.000, 0.000, 0.017, 0.011 and 0.032 respectively).

Chronic (12 week) supplementation with n-3FA, increased the proportion of 20:2n-6, 20:4n-3, AA, eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid ($P$ = 0.005, 0.024, 0.000, 0.000, 0.001 and 0.001 respectively). The higher baseline AA in obese participants is consistent with the literature [1].

We plan to investigate the morphology of the AT and its inflammatory profile by assessing adipocyte size and structure, infiltration of macrophages, angiogenesis, the presence of inflammatory mediators and the expression of inflammatory genes.

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## P-09 Development of new biomarkers for nutritional epidemiology

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To assess the effect of diet on health it is necessary to accurately determine nutrient and food intakes. Traditional dietary assessment methods, such as food frequency questionnaires or 24-h recalls are subjective and they do not consider bioavailability and metabolism. On the other hand, nutritional biomarkers have become a good alternative to estimate dietary intake because they are objective, accurate, consider bioavailability and they are useful to monitor a dietary intervention accomplishment.

Different methods are used to analyze different metabolites in plasma or urine. Liquid chromatography coupled to mass spectrometry is one of the most extensively techniques used although simple colorimetric methods are also useful when less accurate quantification is needed.

We found that total polyphenols in urine are a good biomarker of fruits and vegetables (and their derivatives) consumption and it is very well correlated with polyphenol intake, while isoxanthohumol, a new biomarker developed by our group, is used to accurately measure beer consumption. Other examples are tartaric acid quantification in urine for wine consumption and lycopene and beta-carotene in plasma for tomato products. It is necessary, however, to further investigate new biomarkers for other key foods.

Acknowledgements: These studies have been supported by CICYT (AGL2010-22319-C03, AGL2013-49083-C3-1-R) from the Spanish Ministry of Science and Innovation (MICINN), the *Instituto de Salud Carlos III*, ISCIII (ciberobn-CB06/03, PI1002658, and PI1001407), the European Foundation for Alcohol Research (ERAB, grants EA 1117 and EA 1324), and Generalitat de Catalunya (2014 SGR 773).

## P-10 Weight loss induced by dietary calcium is associated with healthier bone biomarkers in mice

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It has been suggested that the skeleton should be considered an endocrine organ, and that it has a close relationship with energy, glucose and fat metabolism. Studies have shown that both conjugated linoleic acid and calcium have an effect on bone metabolism but, to our knowledge, how this could in turn affect energy metabolism has not been looked into. We have previously shown that supplementation with these two compounds in adult mice fed a high-fat diet has an effect on energy metabolism and can aid weight management. The aim of this study was to assess the effects of these nutrients on bone and energy metabolic markers in tibia. Mice (C57BL/6J) were divided into five groups according to diet and treatment (up to 56 days): control (C), high-fat diet (HF), HF+CLA (CLA), HF+calcium (Ca) and HF with both (CLA+Ca). At the end of treatment, bone formation markers were determined in plasma and expression of selected bone and energy markers was determined in the tibia by qPCR. Initial results show that CLA was associated with decreased tibia weight and minor impact on bone markers. In contrast, Ca supplementation had a significant effect on key players in energy metabolism in bone (increasing both leptin and adiponectin tibia receptors). Furthermore, Ca maintained tibia weight and promoted the expression of bone formation genes such as osteocalcin (Bglap2) and collagen Iα1 (Col1a1). In addition to the weight loss promoting properties of calcium, our results support beneficial effects on bone metabolism in mice.

Acknowledgements: This work was supported by the grant AGL2012-33692 and the EU-funded project (BIOCLAIMS FP7-244995). Our group receives financial support from Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición, CIBERobn. A.C. is supported by a PhD fellowship by the Conselleria d'Educació, Cultura i Universitats, Govern de les Illes Balears, as part of a program cofinanced by the European Social Fund.

## P-11 Maturation of AMP-activated protein kinase in murine skeletal muscle during early postnatal development in obesity-resistant and obesity-prone mice

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AMP-activated protein kinase (AMPK) is engaged in control of energy metabolism. Little is known about changes in AMPK subunits expression and AMPK activity in skeletal muscle during the early postnatal development, between birth and weaning. The aims of the study were to characterize the activity of AMPKα1 and AMPKα2 isoforms and gene expression of these subunits in skeletal muscle while comparing obesity-prone C57BL/6 (B6) and obesity-resistant A/J mice.

Male (M) and female (F) pups of the B6 and A/J mice were born and maintained at 30°C. Gastrocnemius muscle was collected by freeze-clamping and AMPK activity was determined using AMARA peptide substrate. Gene expression was assessed by real-time quantitative RT-PCR.

The activity of AMPKα1 at 10 days (D) was significantly higher in comparison with the AMPKα2 activity in all tested groups. Between 10D and 28D, the AMPKα1 activity decreased in mice of both strains except for A/J F. In A/J mice at 28D, activity of AMPKα2 was higher than that of AMPKα1. Total activity of AMPK (α1+α2) in B6 mice decreased significantly between 10D and 28D but it stayed constant in A/J mice. Expression of AMPKα1 gene was constant in both A/J and B6 mice between 10D and 28D. Expression of AMPKα2 gene increased between 5D and 28D in both strains.

During early postnatal development strain-specific changes in AMPK activity in murine skeletal muscle were observed. While in the obesity-resistant A/J mice the activity stayed constant, it declined in the obesity-prone B6 mice. Changes in AMPK activity in skeletal muscle during early postnatal development may affect propensity to obesity in adulthood, depending on the genetic background of the mice.

## P-12 Early differences in metabolic flexibility between obesity-resistant and obesity-prone mice

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Decreased metabolic flexibility supports development of adverse consequences of obesity. Metabolic flexibility to glucose is traditionally assessed using indirect calorimetry (INCA), which also allows for measuring energy expenditure. The aims of this study were to (i) characterize metabolic flexibility of obesity-resistant A/J and obesity-prone C57BL/6J (B6) mice at weaning, i.e. during the switch from lipid to carbohydrate intake and before the dissociation in body weight; (ii) to compare INCA with glucose tolerance test (GTT) approach.

A/J and B6 mice were maintained at 20°C and weaned to chow diet at 30 days of age. During the first day after weaning, using separate subgroups of 6-hour-fasted mice ($n$=8) of both genders, either GTT (oral, OGTT; and intraperitoneal, IGTT; using 1-3 mg glucose/g body weight, BW) or INCA with either 1 or 7.5 mg glucose/g BW at 34°C were performed. In addition, INCA was also performed during the fasted/re-fed transition at 34°C.

Comparable results were obtained using (i) both OGTT and IGTT with 1 mg glucose/g BW at 20°C; (ii) INCA with 7.5 mg glucose/g BW at 34°C; and (iii) INCA during the fasted/re-fed transition at 34°C. Results indicated lower ability to switch between metabolic substrates associated with low glucose tolerance and relative hyperglycemia in B6 as compared with A/J mice.

We have found lower glucose tolerance using GTT and lower metabolic flexibility using INCA in B6 as compared to A/J mice. These early differences between strains may be linked to the differential genetically-determined propensity to obesity.

## P-13 Plasma acylcarnitines and amino acids levels as an early complex biomarker of propensity to high fat diet-induced obesity

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Induction of obesity by high-fat diet (HFD) in mice is associated with development of impaired glucose tolerance and dyslipidemia, i.e. characteristic features of metabolic syndrome in humans. Various inbred strains of mice vary in their propensity to HFD-induced obesity. Moreover, individual mice differ substantially in their responses to the obesogenic environment, resulting in large variations in body weight gain and glycemic control in spite of identical genetic background. Well characterized C57BL/6J (B6) mice represent a suitable model for studying the mechanisms underlying propensity to obesity and insulin resistance. The aim of this study was to explore whether plasma metabolome, namely the acylcarnitine and amino acids levels, could serve as a biomarker of propensity to obesity and associated disorders.

B6 mice of both genders were weaned to chow diet at 28 days of age. At 3 months of age, all mice were switched to HFD (35% fat, wt/wt). Fasted plasma samples were collected on day after weaning and 22 weeks of age (i.e., after 6 weeks of HFD-feeding). Spearman pairwise correlations between plasma metabolite levels and body weight gain (BWG) were performed with respect to age and gender. The multivariate statistical analyses, such as principal component analysis (PCA) and partial least squares-discrimination analysis (PLS-DA), were adopted to validate the metabolic differences between selected animals with the lowest as well as the highest BWG.

Several weak to moderate correlations were observed. PLS-DA analysis of the plasma metabolites was able to distinguish „low gainers“ from „high gainers“ in male but not in females. Discriminating metabolites differed between week 4 and 22 in male however C18.1OH, free carnitine, Tyr and Orn were in both models.

Our results document that plasma acylcarnitines and amino acids could serve as a gender specific complex biomarker of propensity to obesity and possibly as biomarker of obesity-associated metabolic disorders.

## P-14 Development of the UPLC-MS/MS method for analysis of acylcarnitines and amino acids

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Acylcarnitine (AC) and amino acid (AA) profile analysis is performed for the biochemical screening of metabolic disorders, mainly in newborns. Changes in AC and AA levels in plasma and organs serve as important markers of various metabolic diseases as well as markers of activity of metabolic pathways. Previously, we have developed a method, which allowed us to separate and quantitate AC and AA and even their isobaric species, which is normally problematic. The method was based on liquid chromatography and mass spectrometry. Analysis was performed with HILIC Kinetex column and mobile phase that consisted of water or acetonitrile and buffer at pH 4.00. Using this method, we were able to analyze at about 80 AC and AA. The method was fast and sensitive and it was also validated with good precision and accuracy.

Nowdays, we focused on a more general metabolomic method. Therefore, we have developed a UPLC-MS/MS method, which allows us to analyze 108 metabolites. We use Phenomenex Luna amino column (150x2 mm, 3µm, 100A) and the mobile phase that consists of 20 mM ammonium acetate at pH 9.45 and acetonitrile. A gradient elution takes 12 minutes. During this time, we are able to separate 31 AC, 24 AA, mix of internal standards of AC and AA and 27 metabolites (glucose, cholesterol etc.). The peaks given by this method are more symmetrical than with the previously used method, which helps us to improve area integration.

In summary, we have newly developed a method, which is very efficient, reasonably fast and also sensitive for complex metabolomics analysis of small samples.

## P-15 Biomarkers of adipose tissue health in adulthood are positively affected by moderate resveratrol treatment during lactation in mice

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Increased oxidative metabolism and mitochondrial density are candidate biomarkers of white adipose tissue (WAT) health. Nutrition in early life can program WAT features in adulthood. Resveratrol (RSV) is a food polyphenol with beneficial effects in metabolic health and anti-obesity action in adult animals.

We aim to evaluate long-term effects of RSV treatment during lactation on WAT features, in particular capacity for substrate oxidation and thermogenesis, mitochondrial content and adipocyte size.

Suckling newborn NMRI male mice received a daily oral dose of RSV (2 mg/kg/day) or vehicle, from day 2 to 20 of life. Animals of both groups were weaned onto a chow diet at 21 days, and at day 75 were assigned a normal fat diet (NFD). On day 90, half of the animals of each group (control and RSV) were assigned to a HFD (45% of fat), while the other half remained on the NFD. Weight gain and energy intake was monitored. Animals were sacrificied on day 160. Inguinal WAT (iWAT) was collected and used for gene expression analysis, estimation of mitochondrial DNA content (mtDNA), and morphological and immunohistochemistry analysis.

RSV mice presented a delay in weight gain on a HFD compared to controls, despite their energy intake was higher. RSV mice showed higher mRNA levels in iWAT of beige/brown-like adipocyte and oxidative metabolism markers (Slc27a1, Tmem26, Hoxc9, Prdm16, Ppara, Ppargc1a, Ppargc1b, Pparg, Cpt1b) and of transcription factors specifically related to mitochondria biogenesis and function (Tfb2m, Nrf1, Nrf2). mtDNA content was increased in iWAT of adult RSV mice, which also presented adipocytes of smaller size and increased COXIV immunostaining in iWAT compared to controls. In conclusion, exposure to a moderate dose of RSV during lactation has beneficial effects promoting a healthier phenotype of white fat in adulthood.

Acknowledgments: This work was supported by the Spanish Government (Grant AGL2012-33692, to A.P.) and by a grant from Fundación Ramón Areces (XVI Concurso Nacional, to M.L.B).

## P-16 Glucose-dependent insulinotropic polypeptide (GIP) can serve as a new biomarker of metabolic risk in obesity

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Altered GIP secretion and action was recently linked to obesity-related metabolic disorders but the underlying mechanisms are not well understood. The objective of this study was to compare the pattern of GIP secretion and correlation with metabolic risk markers in obese subjects during fasting and postprandial states.

In obese and non-obese patients fasting and postprandial plasma GIP, glucose, insulin, lipids, glutathione peroxidase, IL-6, sE-selectin, MCP-1, leptin, adiponectin, visfatin were measured in five different time points during oral lipid and glucose tolerance tests.

A total of 114 obese patients and 37 control non-obese subjects, were randomized into the study. Fasting GIP levels differed between obese (32,22 pg/ml) and control (24,27 pg/ml) subjects ($p < 0,05$) and correlated with glucose, triglycerides, total- and LDL-cholesterol, as well as sE-selectin, MCP-1, visfatin and leptin/adiponectin ratio ($p<0,05$). The levels of GIP at 120 min after a high fat meal were significantly higher than those measured at 120 min after glucose ingestion both in obese (365,93 pg/ml vs 156,4 pg/ml, $p<0,05$) as well as control subjects (381,09 pg/ml vs 154,94 pg/ml, $p<0,05$). Enhanced postprandial GIP response to fat or glucose challenge (AUC) positively correlated with glucose AUC, TG AUC, FFA AUC and negatively with glutathione peroxidase activity ($p<0,05$). In patients with the highest fasting GIP concentrations (3$^{rd}$ tertile), increased sE-selectin ($p<0,05$) and MCP-1 blood levels ($p<0,05$) were observed.

We suggest, that GIP is a putative early biomarker of metabolic consequences of obesity and associated proinflammatory state.

Acknowledgements: Study supported by EU FP7 BIOCLAMS, Grant agreement no. 244995, and grant K/ZDS/004497.

## P-17 Identifying scientifically substantiated biomarkers by integrating existing knowledge with results from multiple studies

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The BIOCLAIMS goal is to identify new biomarkers for the effects of food and food components on health, using novel technology such as nutrigenomics. These markers will contribute to the scientific base used for reshaping European legislation on “Health claims made on food”. With this in mind, a strategy was set forth to integrate the BIOCLAIMS project’s study results in a meaningful way.

The objective is the integration of results from multiple BIOCLAIMS consortium studies with existing knowledge, most notably the TNO systems biology department’s knowledge base and expert knowledge from the consortium. Integration is to take place across technologies within studies as well as across studies and organisms. While doing so, provide practically useable integration relevant to European legislation using the EFSA health claims database as a source accepted evidence.

As such, published and unpublished research, biomarker and interaction databases (including EFSA’s), as well as BIOCLAIMS consortium prior research and study designs were chosen as source material. These were systematically mined for information, by machine and by hand. From these mined sources, important biomarkers as well as physiological endpoints were selected to cover the consortium-selected domains of adipose tissue health, metabolic health, and cardiovascular health. Furthermore, interrelationships for these features were also extracted from selected sources. Results from 2 human intervention studies, 1 cross-sectional study and 1 mouse study were also added to the database.

All relevant knowledge and study results were now compiled in a curated database named the ‘BIOCLAIMS Data Integration Website’. The website can now be used to create interactive network visualizations of BIOCLAIMS study results. Separate or aggregated results can now be overlaid and added onto the context most relevant to the goals of BIOCLAIMS. Thus integration of existing knowledge with multiple study results allows for identification of scientifically substantiated new biomarkers.

## P-18 The BIOCLAIMS study at the University of Southampton: The effect of omega-3 fatty acids on metabolic markers in obese and normal weight subjects

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Obesity is an independent factor in the aetiology of cardio-metabolic diseases. It is known that obesity is associated with an exaggerated post-prandial metabolic response and a small number of studies indicate that a similar phenomenon may occur with inflammatory markers.

The aim of this research was to evaluate the differences in fasting and postprandial metabolic markers in obese and normal weight subjects and whether omega-3 fatty acids can induce changes in these responses.

For details of the study design refer to abstracts by Childs CE *et al*. and Paràs-Chàvez C *et al.* Plasma triglyceride, cholesterol, HDL-cholesterol, NEFA and glucose concentrations were measured using an iLAB 600 clinical chemistry analyser using enzyme based kits. LDL-cholesterol concentrations were calculated. Insulin concentrations were evaluated by quantitative sandwich enzyme-linked immunoassay (ELISA) using a commercial kit. Beta cell function and insulin sensitivity were estimated using the homeostasis model assessment (HOMA).

Fasting concentrations of triglycerides, total cholesterol, LDL-cholesterol, glucose, NEFA, insulin and HOMA were all significantly higher in obese compared with normal weight subjects (all $p<0.001$). Moreover, obese subjects showed a higher triglyceride, glucose, and insulin responses to a high fat meal compared with normal weight subjects (all $p<0.001$). Acute and chronic fish oil supplementation resulted in a significant reduction of postprandial triglyceride concentrations in normal weight subjects ($p<0.01$), but not in the obese. Additionally, fasting HDL-cholesterol and HOMA %S were significantly higher after chronic fish oil supplementation in normal weight subjects ($p<0.01$).

Metabolic markers were different between the obese and normal weight subjects. Obesity has a negative effect on post-prandial lipid and glucose handling**.** Fish oil improves metabolic markers in normal weight subjects but obese subjects are resistant to these improvements.

Acknowledgements: We thank Dr Kim Jackson (University of Reading) for her help with the iLAB-based measurements.

## P-19 The BIOCLAIMS study at the University of Southampton: Regulation of Toll-like Receptors (TLRs) by omega-3 fatty acids in obese and normal weight subjects

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Toll like receptors (TLR) are a family of transmembrane proteins involved in the activation of nuclear factor κB and the synthesis of pro-inflammatory cytokines. TLR overexpression has been linked to insulin resistance in mice. Similarly, studies in humans have found higher numbers of blood monocytes expressing TLR4 and TLR2 in diabetic than healthy subjects.

The objective of this research is to explore the effects of obesity, omega-3 fatty acids (O3FA) and a high fat meal on TLR expression on monocytes.

This is a double-bind placebo controlled trial. Healthy normal weight and obese adults were recruited. They made 3 clinic visits. A 6 h postprandial test was performed following consumption of a high fat meal on each visit. On one of the first two visits the meal included O3FA (acute effect). Between the second and third visit subjects consumed O3FA or placebo for 12 weeks (chronic effect). TLR2 and TLR4 expression on $CD14^+$ cells was determined by flow cytometry.

At baseline, normal weight subjects had higher numbers of $CD14^+$ $TLR2^+$ cells, higher TLR2 expression, fewer $CD14^+TLR4^+$ cells and lower TLR4 expression than obese subjects (all $p<0.01$). The high fat meal caused a transient increase on TLR4 expressing monocytes that was greater in obese subjects (all $p<0.01$). Including O3FA with the meal reduced TLR4 expression in obese subjects at 6 h.

The numbers of $CD14^+TLR2^+$ and $CD14^+TLR4^+$ cells and TLR expression levels differ between normal weight and obese subjects and are affected by a high fat meal, particularly in obese subjects. O3FA has limited impact on the postprandial expression of TLRs.

## P-20 The BIOCLAIMS study at the University of Southampton: acute response of plasma inflammatory markers to a high fat meal in normal weight and obese individuals

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Transient changes of inflammatory markers occur after consumption of a meal, particularly one which is high in fat. Obesity is associated with chronic low-grade inflammation and obese subjects may display elevated post-prandial inflammation. Long chain omega-3 (n-3) polyunsaturated fatty acids have anti-inflammatory properties, but their effect on post-prandial inflammation is unknown. Therefore, this study evaluated the acute changes in inflammatory markers after a high-fat meal in normal weight and obese subjects supplemented with 3 g of fish oil (1.8 g of eicosapentaenoic acid and docosahexaenoic acid) or placebo (corn oil).

Cytokines, adhesion molecules and adipokines were measured in plasma of 37 normal weight and 40 obese individuals using microbead-based Luminex kits. These analyses were performed on plasma collected in the fasted state and 1, 2, 3, 4 and 6 hours after a high-fat meal (see poster by Childs et al. for full details).

In the fasted state, obese subjects had higher interleukin (IL)-6, IL-8, C-reactive protein (CRP), vascular endothelial growth factor (VEGF), plasminogen activator inhibitor-1 (PAI-1) and leptin concentrations compared to normal weight individuals. In contrast, concentrations of adiponectin, vascular cell adhesion protein-1 (VCAM-1) and monocyte chemoattractant protein-1(MCP-1) were lower in the obese. The response to the meal was expressed as the area under the concentration curve (AUC). AUC for CRP, leptin, VEGF, and PAI-1 was higher and AUC for IL-10 and adiponectin was lower in obese subjects. Unexpectedly, obese subjects had lower VCAM-1 and MCP-1 AUC. N-3 polyunsaturated fatty acids did not influence the acute inflammatory response to the high-fat meal.

The study confirms low grade inflammation in obesity and demonstrates that obese subjects show a higher level of inflammation in the hours following consumption of a high fat meal. Further investigation will evaluate the effect of chronic n-3 fatty acid supplementation on the response to the high-fat meal.

## P-21 Lysophospholipids: early biomarkers of dyslipidemia?

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The identification of biomarkers as early predictors of disease can enhance clinical diagnosis and help its prevention. In previous studies carried out in our research group, serum lysophospholipids belonging to the categories of lysophosphatidylcholines (lysoPCs), lysophosphatidylinositols (lysoPIs) and lysophosphatidylethanolamines (lysoPEs) were identified as early biomarkers of dyslipidemia. These results were obtained in hamsters fed a high-fat diet and confirmed by a pharmacological model to differentiate diet-consumption markers from dyslipidemia biomarkers. The main objective of the present study was to elucidate if these metabolites were also good early biomarkers in humans. The study was conducted in 90 subjects with different degrees of dyslipidemia based on their low-density lipoprotein cholesterol (LDLc) levels. The exclusion criteria were: hypolipemic treatment, any chronic metabolic disease, triglycerides levels higher than 150 mg/dL, body mass index (BMI) above 30 kg/m2 and/or smokers. Blood samples were taken in over-night fasting conditions to determine some biochemical parameters. Following the American Association of Clinical Endocrinologists (AACE) Guidelines 2012, the control group (CONT, n=30) included subjects with LDLc levels lower than 100 mg/dL; the group of individuals with moderate dyslipidemia (MOD, n=30) had 'near optimal' LDLc levels (100-129 mg/dL); while the levels of the dyslipidemic group (HIGH, n=30) were in the range of 130-189 mg/dL. All groups were balanced by gender. Targeted metabolomics analysis (HPLC-QTOF) was used to quantify lysophospholipid species. The results showed decreased circulating levels of lysoPEs 18:2 and 20:5 in both dyslipidemic groups, while lysoPIs 18:1 and 22:6 were up-regulated only in the HIGH subjects comparing to other groups. Levels of lysoPCs 18:0, 20:0 20:5 and 22:6 were higher in the more developed dyslipidemic group. In conclusion, our results suggest that minor levels of lysoPEs could be early biomarkers of dyslipidemia whereas higher lysoPCs and lysoPIs levels might indicate a later state of the disease.

Acknowledgements:The research leading to these results received funding from ACC1Ó (TECRD12-1-0005) and from the European Union's Seventh Framework Programme FP7 2007-2013 under grant agreement nº 244995 (BIOCLAIMS Project). Susana Suárez is also grateful to Government of Catalonia for the grant received (FI-DGR 2014).

## P-22 The BIOCLAIMS study at the University of Southampton: a human high-fat dietary challenge to assess the acute and chronic effects of omega-3 fatty acid supplementation

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The BIOCLAIMS Study at the University of Southampton assessed the acute and chronic effects of omega-3 fatty acid supplementation upon the post-prandial metabolic and inflammatory responses to a high-fat dietary challenge.

100 healthy adults were recruited from the Southampton area between February 2012 and October 2013; this included 50 adults with a BMI of 18.5-25, and 50 adults with a BMI 30-40 kg/$m^2$ with waist circumference > 94cm for men > 80cm for women. These volunteers attended for three high-fat dietary challenges, with blood samples collected during the 6 hours after the meal. At challenge day 1 and 3, an adipose tissue biopsy was collected. At each challenge day, volunteers arrived having fasted overnight, anthropometric measures were made, a baseline blood sample was collected, and a standardised high-fat breakfast was provided. Volunteers were provided with three 1 g capsules to take with their breakfast.

The acute study: During challenge day 1 and 2, volunteers were randomly assigned to receive either 3 x 1 g fish oil capsules (EPAX6000, providing 1.9 g EPA + DHA), or 3 x 1 g corn oil capsules (providing 1.6 g LA, 0.9 g OA).

The chronic study: Following challenge day 2, volunteers were randomised to receive either fish oil or the corn oil placebo to be taken daily for three months, before returning for challenge day 3. At challenge day 3, all participants were provided with the standardised breakfast as before, and provided with 3 x 1 g corn oil capsules (providing 1.6 g LA, 0.9 g OA) to take with their breakfast.

While the primary outcome of this study is the effect of acute and chronic omega-3 fatty acid supplementation upon inflammatory markers (see poster by de Castro et al.), numerous secondary outcome measures are under assessment, including collaborations with partner organisations within the BIOCLAIMS consortium. These include the effect upon adipose tissue composition (see poster by Fisk et al.), post-prandial plasma lipid responses (see poster by West et al), metabolic markers (see poster by Paràs-Chàvez et al.), TLR expression (see poster by Paràs-Chàvez et al.) and effects upon gut hormones (see poster by Yeh et al).

## P-23 The BIOCLAIMS study at the University of Southampton: the effect of chronic omega-3 fatty acid supplementation upon circulating gut hormones

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The BIOCLAIMS Study at the University of Southampton assessed the acute and chronic effects of omega-3 fatty acid supplementation upon the post-prandial metabolic and inflammatory responses to a high-fat dietary challenge (see poster by Childs et al. for full details of the study design).

Circulating gut hormones were assessed in the post-prandial period after chronic omega-3 supplementation in a sub-set of study participants (n = 58). Eight hormones were assessed in protease inhibitor treated plasma: amylin, ghrelin, gastric inhibitory polypeptide (GIP), glucagon-like peptide-1 (GLP-1), insulin, leptin, pancreatic polypeptide (PP) and peptide YY (PYY). Fasted hormone status among lean and obese adults was compared by t-test, and incremental area under the curve data from a high fat meal challenge was analysed by two-way ANOVA to assess the effect of obesity status and fish oil treatment, controlled for participant age.

Obesity status had a significant effect upon baseline fasting plasma hormone concentrations, with higher baseline ghrelin among lean participants ($p < 0.001$) and higher baseline insulin and leptin among obese participants ($p < 0.001$).

Post-prandial insulin and leptin responses were higher among obese participants ($p<0.001$, $p = 0.044$). A significant effect of fish oil treatment was observed in the post-prandial PP response ($p = 0.022$). A significant interaction between obesity status and fish oil treatment was observed upon post-prandial ghrelin ($p = 0.017$). No significant effects of obesity status or fish oil treatment were observed upon the post-prandial response of amylin, GIP, GLP-1 or PYY.

These data indicate that healthy obese subjects have an altered fasting gut hormone status compared to normal weight individuals, and generate an altered post-prandial hormone response to a high-fat meal challenge which can be influence by providing fish oil treatment with the meal. Further analysis will be conducted to determine whether these changes in circulating gut hormones correlate to changes in inflammatory markers.

## P-24 "Vivir Mejor": An online program for promoting healthy lifestyle and weight loss in overweight and obese adults with hypertension.

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The prevalence of overweight and obesity is on the rise worldwide with severe physical and psychosocial consequences. Hypertension is one of the comorbidities associated with obesity. Changes in lifestyles through eating behavior and physical activity level are the critical components in the prevention and treatment for hypertension and obesity. Data from several studies indicate that the usual procedures to promote these healthy habits in health services are inadequate. ICTs has been demonstrated as an effective tool for implementation of psychological interventions focused on this type of population. This study aims to describe a totally self-applied online program (¨*Vivir Mejor*¨) to promote healthy lifestyles (eating behavior and physical activity) for obese participants with hypertension.

Participants (BMI=25-35) will be recruited from users of a hypertension unit of a public hospital and will be randomized into two groups: experimental group *"Vivir Mejor"* and control group (treatment as usual). *"Vivir Mejor"* program is composed by 9 modules aimed for promoting healthy eating habits and increase physical activity. Modules will be sent via Internet periodically. After 6 months, a follow-up testing phase and a tracking module will be set. The outcomes variables will include anthropometric data, changes in eating behavior and physical performance and cardiovascular variables.

It is expected that online program will show efficacy, promoting changes in specific variables like healthy eating behavior, physical activity and medical variables, compared to treatment as usual. Moreover, it is expected that participants will accept and assess the online program positively.

The study and use of ICT's for improving lifestyle and treat obesity will have important implications in terms of costs, efficacy and the number of beneficiaries.

Acknowledgement: CIBERobn Fisiopatología de la Obesidad y Nutrición.

## P-25 Leptin supplementation during lactation induces hypothalamic expression of key metabolic and neuronal health biomarkers in young and exercise-trained adult female mice

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Leptin treatment during lactation affects the balance of hypothalamic signals, such as appetite neuropeptides in adult rats. Other hypothalamic signal, brain-derived neurotrophic factor (BDNF) is also related with energy control, and with cognitive function. We aimed to study the effects of leptin supplementation during lactation on the expression of these (and related) molecules in the hypothalamus of young and exercise-exposed adult female mice.

Suckling newborn NMRI female mice were supplemented with physiological dose of leptin or vehicle (control) from day 2 to day 20 of life. Part of both control and leptin animals was sacrificed at the age of 5 weeks. Remaining animals were subdivided into two groups at the age of 11 weeks, whether regular exercise was imposed (30´ of daily swimming) or not, until sacrifice at 6-month age. Hypothalamus from young and adult mice was collected. Biometrical parameters were routinely monitored.

Cumulative caloric intake (significantly) and body fat (a tendency) were lower in the leptin group in young animals. Regular swimming caused decreased fat content and food intake (in both control and leptin groups). Hypothalamic BDNF, PGC1α (peroxisome proliferator-activated receptor γ co-activator 1α), FNDC5 (fibronectin type III domain-containing protein 5) and Sirt1 (sirtuin 1) mRNA levels were upregulated in young leptin-treated females; while there were no differences in the mRNA levels of ObRb (leptin receptor) and any of the anorexigenic and orexigenic neuropeptides, namely: CART (cocaine and amphetamine-regulated transcript), NPY (neuropeptide Y), AgRP (agouti-related protein) and POMC (pro-opiomelanocortin). On the other hand, adult females showed upregulation of BDNF, Sirt1, ObRb and all neuropeptides when exposed to exercise only in the leptin group.

These findings support the metabolic imprinting potential of early leptin supplementation and show its interaction with physical activity. Moreover, the young animals may be suitable for the search and/or validation of health biomarkers.

Acknowledgments: This work was supported by the Spanish Government (Grant AGL2012-33692, to A.P.).

## P-26 Pectin supplementation reduces risk of cardiovascular disease in obesity-prone rat model

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Nowadays microbiota has been identified as a possible novel factor able to modulate cardiovascular risk and can be modified by prebiotics.

We aimed to study the effects of supplementation with a prebiotic (high esterified pectin (HEP)), in the obesity-prone rat model CR (offspring of moderate caloric-restricted dams), to reduce cardiovascular risk. After weaning, male offspring of 20% caloric-restricted dams (pregnancy days 1-12) was fed with a standard diet (CR) or supplemented with 10% HEP (CRP) until 4-month age; then half of each group was also supplemented with 30% sucrose (CRS/CRPS). Control groups (C/CS) were also studied.

At 5-months, blood pressure (BP) was measured. Animals were sacrificed at 6-month age and heart was weighted and collected. Serum level of FGF21 was also determined. Heart mRNA expression levels of key genes *Cpt1*b, *Ampka2* (oxidation), *Fasn* (lipogenesis), *Fgfr1*, *Klb* (FGF signal transduction) and *Nppa*, *Nppb* (natriuretic peptides) were determined by RT-qPCR.

HEP supplementation reduced significantly BP levels. Total lipids and TG in heart were lower in HEP animals under chow and sucrose diets. HEP supplemented animals were able to increase mRNA expression of Cpt1b and Ampka, and maintain Fasn levels under sucrose stimulus. These results were accompanied by higher levels of FGF21 in serum followed by an increment of mRNA levels of its specific receptor Klb in HEP animals. Moreover, Nppa and Nppb mRNA expression levels were higher in HEP animals under both diets.

Altogether, these results suggest that HEP supplementation in CR animals can reduce cardiovascular risk factors, as BP and lipid content in heart, as well as to promote lipid oxidative capacity under high-sucrose diet. An activation of FGF21 signalling may be involved in the response observed, as well as the action of natriuretic peptides. Moreover, the expression of some of the molecules studied can be analysed as potential cardiovascular health biomarkers.

## P-27 *Cpt1a* expression in PBMC as an interesting biomarker of diet-related metabolic alterations and metabolic recovery

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Peripheral blood mononuclear cells (PBMC) constitute a useful biological material for nutrigenomic studies as they can reflect metabolic response to different diets, nutritional compounds or feeding conditions. *Cpt1a* is a beta-oxidation gene whose expression is highly regulated by diet composition in key energy-homeostasis tissues. Here, we aimed to identify if *Cpt1a* expression was altered in PBMC in response to the intake of unbalanced diets. To that purpose, we analysed by real-time RT-PCR *Cpt1a* gene expression in PBMC samples of control rats, in rats fed *ad libitum* with a cafeteria diet, a commercial high fat diet or a control diet after the intake of a cafeteria diet (post-cafeteria model), and in rats fed a commercial high fat diet and a high protein diet administered in isocaloric conditions (pair-feeding) to control animals. Dietary treatment was performed in adult animals for 4 months, and PBMC samples were obtained and analysed monthly. Cafeteria diet intake resulted in important overweight and related complications while the intake of a commercial high fat diet produced metabolic alterations but in the absence of increased body weight. *Cpt1a* expression increased as result of the intake of both obesogenic diets even in an early stage of dietary treatment, and its levels were recovered after withdrawal of the cafeteria diet. Additionally, *Cpt1a* mRNA expression was also increased in PBMC of animals which were pair-fed with unbalanced diets rich in fat or protein, both of them linked to metabolic disturbances. Thus, we propose analysis of *Cpt1a* expression in PBMC as a good biomarker to detect early metabolic alterations caused by the consumption of unbalanced diets, even in the absence of increased body weight, as well as a marker of metabolic recovery associated to weight loss.

Acknowledgements: CIBER de Fisiopatología de la Obesidad y Nutrición is an initiative of the ISCIII. This work was supported by the EU FP7 project BIOCLAIMS -FP7-244995. Laboratory of Molecular Biology, Nutrition and Biotechnology is a member of the European Research Network of Excellence NuGO.

## P-28 Dietary control before gestation in rats made obese by cafeteria diet feeding apparently avoids detrimental effects in their offspring

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It is generally recognized that maternal obesity or maternal exposure to an excess of calories during gestation and/or lactation have negative effects in the late metabolic health of the offspring. Here we analysed whether removal of the obesogenic diet before mating in female rats made obese by feeding a cafeteria diet may avoid the detrimental effects of maternal obesity on the offspring body weight and the expression of energy homeostasis related genes in key tissues. For this purpose, female rats fed a cafeteria diet from days 10 to 100 of life and then a standard diet for one month (postcafeteria dams) and female control rats were mated with male rats. The male offspring of both control and postcafeteria dams were killed at postnatal day 26 under *ad libitum* feeding conditions or 14 h fasting. The expression of key genes related to lipid metabolism in liver and retroperitoneal white adipose tissue (rWAT) were analysed. At this juvenile age, offspring of postcafeteria dams presented lower body weight than controls, but showed no differences concerning body fat content. They showed no differences regarding the expression of key genes involved in hepatic lipid metabolism compared to controls, and a similar response to fasting conditions in terms of gene expression of key lipogenic and lipolitic genes in rWAT and liver. However, offspring of postcafeteria dams displayed a lower expression of lipogenic (Pparg, Srebf1, Fasn) and lipolitic (Pnpla2) genes in rWAT compared to controls. These results suggest that removal of a cafeteria diet before gestation, although without complete reversion of body weight excess, may apparently avoid detrimental effects of maternal obesity on the hepatic metabolism of their offspring, although is associated to changes at gene expression level in rWAT at a juvenile age that could affect their future metabolic health.

Acknowledgements: The research leading to these results has been funded by the Spanish Government (AGL2012-33692), and the Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición, CIBEROBN.

## P-29 Cafeteria diet intake during lactation in rats affects the offspring capacity to regulate energy homeostasis

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The suckling period is a critical phase for the developmental programming of later susceptibility to chronic disorders and diseases, such as obesity and related metabolic alterations. Here, we aimed to assess, in rats, the influence of maternal intake of a cafeteria diet during lactation on the metabolic response to fed/fasting conditions in key tissues involved in energy homeostasis in their male offspring at weaning. Dams were fed a standard chow (control) diet or a cafeteria diet throughout lactation. At weaning, male offspring from control and cafeteria fed dams were killed under *ad libitum* feeding conditions and after 14-h fasting. Blood parameters were measured and the expression of key genes related to energy metabolism in liver, inguinal white adipose tissue (iWAT) and hypothalamus were analysed. At weaning, male offspring of cafeteria fed dams showed lower body weight than controls, but they presented greater fat content. They also displayed higher circulating levels of leptin and triglycerides, and of free fatty acids under feeding conditions. Concerning gene expression, they also showed: i) an altered fed/fasting expression profile of Npy and Pomc in hypothalamus; ii) lower hepatic expression of genes related with lipogenesis (Srebf1, Fasn, Scd1) and higher expression of fatty acid oxidation-related genes (Ppara, Cpt1a), specially under feeding conditions; and iii) lower mRNA levels of lipogenesis-related genes (Srebf1, Fasn) under feeding conditions and higher expression levels of leptin and genes related with fatty acid uptake (Cd36 and Lpl), and lipolysis (Pnpla2) in iWAT. These results suggest that maternal intake of a cafeteria diet during lactation affects energy homeostasis of the offspring, associated to increased circulating leptin levels and an impaired response to fed/fasting conditions, which may predispose to metabolic alterations in adulthood.

Acknowledgements: The research leading to these results has been funded by the Spanish Government (AGL2012-33692), and the Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición, CIBEROBN.

## P-30 MyNewGut: Microbiome's influence on energy balance and brain development/function put into action to tackle diet-related diseases and behaviour

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Emerging evidence suggests that the human gut microbiota and its genome (microbiome) play diverse physiological roles that influence our health and wellbeing. The microbiome contributes to regulating energy balance, brain development and function, thereby determining our risk of developing brain and diet-related disorders. The MyNewGut project, which receives funding from the European Union's 7th FP, will research how the human gut microbiota and its genome influence obesity, behavioural- and lifestyle-related disorders and vice versa. The 5-years project will work towards identifying the influence of modifiable lifestyle factors on the gut microbiome and the specific intestinal bacteria that contribute to and predict these disorders at critical stages in life. This information will ultimately be translated into practical applications, including the development of more effective nutritional interventions and microbiome-based dietary recommendations targeting the gut ecosystem to prevent these disorders.

The MyNewGut project will provide a unique platform that will enable understanding of the extent to which microbiome-related features are modifiable through dietary strategies. This will be possible by conducting tightly controlled epidemiological and human intervention studies, as well as assessing a combination of classical robust physiological outcomes with high-throughput omics-technologies, and applying computational modelling and systems biology.

MyNewGut also aims to strengthen the competitiveness of the EU food industry and SMEs by providing robust scientific evidence that a balanced and healthy diet containing foods and ingredients, targeting the gut microbiota, can help control the prevalence of obesity and associated metabolic and behavioural disorders. Such robust scientific evidence will enable industry to provide foods with reliable health claims that can contribute to healthier diets and the long-term health of consumers contributing to the development of new strategies and EU policies on public health.

## P-31 The BIOCLAIMS study at the University of Southampton: the effect of chronic omega-3 fatty acid supplementation upon antioxidant status and markers of oxidative stress

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The BIOCLAIMS Study at the University of Southampton assessed the acute and chronic effects of omega-3 fatty acid supplementation upon the post-prandial metabolic and inflammatory responses to a high-fat dietary challenge (see poster by Childs et al. for full details of the study design).

Antioxidant status and markers of oxidative stress were assessed at baseline and after chronic fatty acid supplementation in a sub-set of study participants (n = 33). Human mercaptalbumin (HMA), human non-mercaptalbumin (HNA1, HNA2), malondialdehyde, lutein/zeaxanthin, retinol, tocopherols (α, γ, δ), β-cryptoxanthin, lycopene, and α- and β-carotene were measured in plasma. Data were compared between normal weight and obese subjects by t-test, and change after chronic supplementation was analysed by two-way ANOVA to assess the effect of obesity status and omega-3 fatty acid treatment.

Obese subjects had higher concentrations of γ- and δ-tocopherol than normal weight subjects ($p = 0.0025$, $p = 0.001$). Chronic n-3 fatty acid supplementation resulted in an increased percentage of HMA and a reduced percentage of HNA1 in both lean and obese participants, though this effect was significantly greater among normal weight participants ($p < 0.05$). Significant interactions between body composition and n-3 PUFA treatment were observed upon γ- and δ-tocopherol status. Normal weight participants had increased γ- and δ-tocopherol after chronic fatty acid supplementation, with these increases highest among those receiving corn oil supplementation. In obese participants, increasing γ- and δ-tocopherol status was only observed among those receiving fish oil.

These data demonstrate that there are significant differences in some antioxidant vitamins between healthy normal weight and obese adults, and that normal weight and obese participants have differing responses to chronic dietary fatty acid supplementation. Further analysis will be conducted to determine whether the changes in antioxidant status relate to changes in inflammatory markers or immune function.

Acknowledgements: The research leading to these results has received funding from the European Union's Seventh Framework Programme FP7 2007-2013 under grant agreement nº 244995 (BIOCLAIMS Project) and the Federal Ministry of Science, Research and Economy of Austria.

## P-32 Assessment of metabolic flexibility of old and adult mice using three non-2 invasive, indirect calorimetry-based treatments

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Indirect calorimetry (InCa) can potentially be used to non-invasively assess metabolic and 2 age-related flexibility. To assess the use of InCa for this purpose, we tested the sensitivity and 3 response stability over time of three InCa-based treatments in old versus adult mice. Diurnal 4 patterns of respiratory exchange ratio were followed for 24 hours under standard conditions 5 (Treatment 1), but the results were not stable between test periods. As a challenge, fasted 6 mice received glucose to test switch-effectiveness from fat to glucose oxidation (Treatment 7 2). No differences between groups were observed, although old mice showed higher adiposity 8 and lower white adipose tissue mitochondrial density, indicative of age-impaired metabolic 9 health. Lastly, adaptation to a challenge of oxygen restriction (OxR, 14.5 % O2) was assessed 10 as a novel approach (Treatment 3). This treatment stably detected significant differences: old 11 mice did not maintain reduced oxygen consumption under OxR during both test periods, 12 while adult mice did. Further biochemical and gene expression analyses showed that OxR 13 affected glucose and lactate homeostasis in liver and WAT of adult mice, supporting the 14 observed differences in oxygen consumption. 15

In conclusion, InCa analysis of the response to OxR in mice is a sensitive and reproducible 16 treatment to non-invasively measure age-impaired metabolic health.

Acknowledgements: This work was supported by the European Union's Seventh Framework Program FP7 2007-2013 under grant agreement no. 244995 (BIOCLAIMS Project).

This work has been published in: Journal of Gerontology Biological Sciences DOI: 10.1093/ gerona/glu027.

## P-33 Oxygen restriction as challenge test reveals early high-fat-diet-induced changes in glucose and lipid metabolism

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Challenge tests stress homeostasis and may reveal deviations in health that remain masked under unchallenged conditions. Ideally, challenge tests are non-invasive and applicable in an early phase of an animal experiment. Oxygen restriction (OxR; based on ambient, mild normobaric hypoxia) is a non-invasive challenge test that measures the flexibility to adapt metabolism. Metabolic inflexibility is one of the hallmarks of the metabolic syndrome. To test whether OxR can be used to reveal early diet-induced health effects, we exposed mice to a low-fat (LF) or high-fat (HF) diet for only 5 days. The response to OxR was assessed by calorimetric measurements, followed by analysis of gene expression in liver and epididymal white adipose tissue (eWAT) and serum markers for e.g. protein glycation and oxidation. Although HF feeding increased body weight, HF and LF mice did not differ in indirect calorimetric values under normoxic conditions and in a fasting state. Exposure to OxR; however, increased oxygen consumption and lipid oxidation in HF mice versus LF mice. Furthermore, OxR induced gluconeogenesis and an antioxidant response in the liver of HF mice, whereas it induced de novo lipogenesis and an antioxidant response in eWAT of LF mice, indicating that HF and LF mice differed in their adaptation to OxR. OxR also increased serum markers of protein glycation and oxidation in HF mice, whereas these changes were absent in LF mice. Cumulatively, OxR is a promising new method to test food products on potential beneficial effects for human health.Assessment of metabolic flexibility of old and adult mice using three non-2 invasive, indirect calorimetry-based treatments.

Acknowledgements: This work was supported by the European Union's Seventh Framework Program FP7 2007-2013 under grant agreement no. 244995 (BIOCLAIMS Project).

This work has been published in: Pflügers Archiv - European Journal of Physiology, DOI 10.1007/s00424-014-1553-8.

## P-34 Metabolic adaptation of white adipose tissue to acute short-term oxygen restriction in mice

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White adipose tissue (WAT) expansion during e.g. the development of obesity reduces oxygen availability in WAT. Little is known on the adaptation of WAT to mild oxygen restriction (OxR). Therefore, we studied metabolic adaptation to acute OxR in fasted, diet-induced moderately obese mice that were exposed to mild hypoxic (12% O2) or normoxic (20.9% O2) conditions for 6 hours.

Adaptation to OxR was assessed by determination of amino acid and (acyl)carnitine levels in serum and WAT and by whole genome expression analysis in WAT. Adaptation was also assessed during the exposure with indirect calorimetry.

OxR reduced mitochondrial oxidation at whole-body level, as shown by a reduction in whole-body oxygen consumption and an increase in serum long-chain acylcarnitine levels. WAT did not seem to contribute to this serum profile since only short-chain acylcarnitines were increased in WAT and gene expression analysis indicated an increase in mitochondrial oxidation. In addition, OxR did not induce oxidative stress in WAT, but increased molecular pathways involved in cell growth and proliferation. OxR increased levels of the amino acids tyrosine, lysine and ornithine in serum and of leucine/isoleucine in WAT.

This study shows that OxR limits oxidative phosphorylation at whole-body level, but in WAT compensatory mechanisms seem to operate. The down-regulation of the mitochondria-related genes may be considered as a biomarker profile for WAT mitochondrial reprogramming in response to acute exposure to limited oxygen availability

Acknowledgements: This work was supported by the European Union's Seventh Framework Program FP7 2007-2013 under grant agreement no. 244995 (BIOCLAIMS Project).

## P-35 Direct comparison of the flavonoids quercetin, hesperetin, epicatechin, apigenin and anthocyanins on metabolic health effects in high-fat diet fed mice

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Dietary flavonoid intake is associated with reduced risk of cardiovascular diseases, possibly by affecting metabolic health. The relative potency of different flavonoids in causing beneficial effects on energy and lipid metabolism has not been investigated.

Effects of quercetin, hesperetin, epicatechin, apigenin and anthocyanins, in mice fed a high-fat diet (HF) for 12 weeks were compared, relative to normal-fat diet. HF-induced body weight gain was significantly lowered by all flavonoids (17-29%), but most by quercetin. Quercetin significantly lowered HF-induced hepatic lipid accumulation (71%). Mesenteric adipose tissue weight and serum leptin levels were significantly lowered by quercetin, hesperetin, and anthocyanins. Adipocyte cell size and adipose tissue inflammation were not affected.

The effect on body weight and composition could not be explained by individual significant effects on energy intake, energy expenditure or activity. Lipid metabolism was not changed as measured by indirect calorimetry or expression of known lipid metabolic genes in liver and white adipose tissue. Hepatic expression of Cyp2b9 was strongly down regulated by all flavonoids.

In conclusion, all flavonoids lowered parameters of HF-induced adiposity, with quercetin being most effective. Hepatic Cyp2b9 expression is a marker for a possible common mode of action of these flavonoids. Serum leptin levels can be used as a sensitive marker for the flavonoid effects on HF-induced adiposity.

## P-36 Pectin supplementation for one month in adult rats ameliorates age-related impairment in peripheral insulin and leptin sensitivity

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Dietary fiber has been shown to exert beneficial effects on body weight management and obesity-related metabolic alterations. Here, we aimed to assess whether pectin supplementation for one month in adult rats is able to ameliorate age-associated disturbances in peripheral insulin and leptin actions. 7-month-old male Wistar rats were used. They were distributed into three groups: control (rats fed *ad libitum* a standard-diet), pectin (rats fed *ad libitum* a standard-diet supplemented with 10% pectin, w/w), and pair-fed (rats pair-fed to the pectin group). Body weight, fat content and food intake were followed. One month after dietary intervention, animals were sacrificed under feeding conditions to obtain tissue samples. Blood samples were also obtained under feeding and fasting conditions before and after dietary intervention. Gene expression and protein analyses in WAT and liver were undertaken. Both pectin and pair-fed rats ate fewer calories and showed lower body-weight gain than controls. Both groups also underwent a decrease in leptin levels (particularly the pectin group) and an increase in adiponectin levels (particularly the pair-fed group) after dietary intervention. Notably, pectin-treated animals, but not pair-fed animals, showed lower body fat content and HOMA-IR after dietary intervention. At the molecular level, compared to controls, pectin-treated rats showed a widespread decline in the expression levels of genes related to energy uptake and lipogenesis in WAT and liver, which was not evidenced in the pair-fed group. These effects appear to be associated with improved leptin signaling, as evidenced by the higher levels of total and phosphorylated STAT3 in these tissues, despite the diminished plasma leptin levels. These results suggest that 10% pectin supplementation for one month in adult male rats decreases body fat content and ameliorates age-related insulin and leptin resistance, independently or at least more acutely than what could be attributed to the decrease in energy intake, overall contributing to better metabolic health.

Acknowledgments: The research leading to these results was supported by the Spanish Government (grant AGL2012-33692), and the Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición, CIBERobn. Authors belong to the Nutrigenomics-group at UIB, awarded as 'Group of Excellence' of the Autonomous Community of the Balearic Islands (CAIB) and supported by 'Direcció General d'Universitats, Recerca i Transferència del Coneixement' of Regional Government (CAIB) and FEDER funds (EU).

## P-37 Expression of *Mest* and *Lep* genes in whole blood as predictive biomarkers of adipose tissue expansion sensitive to food bioactives

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Early biomarkers of adipose tissue expansion that are predictive of fat accumulation in the long term are of interest, particularly those in easily accessible samples. *Mest* and *Lep* gene expression levels in adipose tissue are candidate biomarkers of tissue expansion following obesogenic diets. Resveratrol and hydroxytyrosol are bioactive food compounds shown to counteract responses to obesogenic diets.

We aimed to investigate whether changes in the expression of *Mest* and *Lep* genes in whole blood may function as early predictive biomarkers of future adipose tissue expansion under an obesogenic diet.

A 21 day dietary intervention experiment was designed in male C57BL16 mice. Four groups were used: a control group fed a normal fat diet (NF group), a group fed a 45% high fat diet (HFD) and treated with vehicle, and groups fed the HFD and treated with resveratrol (30 mg/kg bw/day) or hydroxytyrosol (20 mg/kg bw/day), respectively. Morphometric parameters, food intake, and the mRNA expression levels of *Mest* and *Lep* in epididymal white adipose tissue (eWAT) at day 21 and in whole blood at days 5 and 21 of the experiment were analyzed.

There were no differences between groups in energy intake. The three HFD-fed groups gained more weight and fat mass than the NF group. *Mest* and *Lep* were overexpressed in eWAT of mice of the HFD-fed groups. Hydroxytyrosol-treated mice showed lower mRNA expression levels of *Mest* in whole blood at day 5 and won less visceral fat mass under the obesogenic diet compared to the vehicle-treated HFD-fed mice, as indicated by the subcutaneus/visceral fat index at the end of the experiment. *Mest* expression in total blood at day 5 was positively correlated with adiposity and negatively with subcutaneous/visceral fat index at the end of the experiment. *Lep* mRNA was not consistently detectable in whole blood by qPCR.

In conclusion, early changes under an obesogenic diet in *Mest* mRNA expression in whole blood may function as a predictive marker of future fat accumulation, and be useful in the screening and evaluation of bioactive compounds with potential anti-obesity action.

Acknowledgements: Supported by European Union's Seventh Framework Program FP7 2007-2013 under grant agreement nº 244995 (BIOCLAIMS Project).

## P-38 DHA from optional to mandatory nutrient in infant formulae; the importance of innovation and communication

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Research on DHA for visual and brain development in infants started more than 25 years ago. As is normal with every ingredient at the beginning of innovation, the science cannot meet the extremely high level required for a positive health claim assessment according to the European health claim legislation, which is the most stringent around the globe. However, that should not preclude that the presence and level of such an ingredient could be communicated. To stimulate a healthy competitive innovation process in the EU with European SME, academia & investment in research, it is important that the presence and level of the innovative ingredient can be indicated on the label. At the beginning of innovation, the key question to answer is: “is the innovative ingredient safe and suitable for the intended population under the proposed condition of use”? It is unnecessarily restricting innovation if the level and presence of an innovative ingredient can only be mentioned on a label when the efficacy is proven beyond any doubt, since this can take up to 20 years of research whereas safety, suitability and expected benefit can already be demonstrated after 5 years. The possibility to communicate on the presence and level of this ingredient has resulted into continued investments in collaborative research with academia and SME. After 25 years of research, with numerous clinical studies, the claim ‘DHA supports the normal visual development of infants’ was permitted as the first European health claim on formulae for infants. Now, after the assessment by EFSA, DHA is recommended as a mandatory nutrient for infants. Innovation of ingredients that are ‘optional’, or sometimes even called ‘unnecessary’, may prove to be mandatory nutrients 25 years from now. The possibility to communicate on new ingredients is a way to drive innovation, research investments, and nutritional improvements.

## P-39 Involvement of skeletal muscle in the pterostilbene-induced amelioration of glycaemic control in obese rats

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Pterostilbene is a polyphenol chemically related to resveratrol. It shows higher bioavailability and longer half-life than resveratrol. In a previous study, we demonstrated that pterostilbene improved glycaemic control due to an increased glucose uptake. The present study aimed to determine the involvement of skeletal muscle in the effect of pterostilbene on the amelioration of the glycaemic control alterations induced by an obesogenic feeding.

Twenty-seven male growing Wistar rats were divided into three experimental groups (n=9), and fed an obesogenic diet, supplemented or not (control group; C group) with pterostilbene: PT15 group (15 mg pterostilbene/kg body weight/d), PT30 group (30 mg pterostilbene/kg body weight/d). At the end of the experimental period (6 weeks), rats were sacrificed and gastrocnemius muscle was dissected. Triacylglyceride and cardiotophin-1, a key regulator of glucose homeostasis, content in skeletal muscle were measured spectrophotometrically. Glucose transporter GLUT-4 and the phosphorylated protein kinase B to total protein kinase B ratio (p-Akt/total-Akt) protein expressions were determined by western-blot.

Triacylglyceride content was not modified by pterostilbene treatment. Further, cardiotrophin-1 was increased ($P<0.05$) in PT30 group, but not in PT15 group. As far as protein expression of the glucose transporter GLUT4 is concerned, rats from both pterostilbene-treated groups showed significantly higher values than those from the control group. No significant differences between the two groups treated with pterostilbene were observed. Again, in PT30 group, but not in PT15 group, the p-Akt/total Akt ratio was significantly increased ($P<0.05$). Consequently, it can be supposed that in this group GLUT4 was more efficiently translocated to the membrane.

Summing up, pterostilbene can be considered as a promising molecule for improving glycaemic control. Indeed, an increase in skeletal muscle glucose uptake, but not the reduction in its triacylglyceride content, seems to be involved in the anti-diabetic effect of this phenolic compound.

Acknowledgements: Research relating to this abstract was funded by Ministerio de Economía y Competitividad (AGL2011-27406-ALI), Instituto de Salud Carlos III (CIBERObn), Government of the Basque Country (IT-512-13) and University of the Basque Country (UPV/EHU) (ELDUNANOTEK UFI11/32). S. Gómez-Zorita is a recipient of a doctoral fellowship from the University of the Basque Country (UPV/EHU). Authors have no conflicts of interest.

## P-40 The liver delipidating effect induced by a combination of resveratrol and quercetin is not mediated by increased mitochondriogenesis

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In a previous experiment, a reduction in liver fat content was shown when two polyphenols, such as resveratrol (RSV), found in grapes and wine, and Quercetin (Q), found in onions and kales, were administered together in rats. This effect was considered synergistic due to the fact that neither RSV nor Q separately administered affected hepatic fat content. One of the mechanisms involved was the fatty acid oxidation, which was increased in RSV+Q supplemented group.

The aim of this work was to analyze whether the increased fatty acid oxidation was mediated by a stimulation of mitochondriogenesis induced by RSV+Q.

Fourty Wistar rats were divided into four groups fed an obesogenic diet during 6 weeks: control rats (C), rats treated with resveratrol (RSV; 15 mg/kg/d), quercetin (Q; 30 mg/kg/d) and both molecules (RSV+Q). Enzymatic activities of citrate synthase as well as gene expression of PPARα, NFR-1 and TFAM were assessed by spectrophotometry and RT-qPCR methods respectively. The results were analyzed by ANOVA I and post-hoc Newman Keuls test.

Citrate synthase, a marker of mitochondrial density, remained unchanged in all groups. In this line, no significant changes were observed in the expression of PPARα, NRF-1 and TFAM, three genes involved in the control of mitochondriogenesis.

In conclusión, the previous synergistic effect observed in liver delipidation when resveratrol and quercetin are administered together, which is mediated by increased fatty acid oxidation, is not associated with increased mitochondriogenesis.

Acknowledgements: This study has been supported by Instituto de Salud Carlos III (CIBERObn and RETIC PREDIMED, Ministerio de Economía y Competitividad (AGL2011-27406-ALI), Gobierno Vasco (GIC07/120-IT-265-07) and University of the Basque Country (UPV/EHU) (UFI 11/32). Authors do not have conflict of interest.

## P-41 The genetic variant rs16147 in NPY may contribute to explain the differential effect of high or low adherence to the Mediterranean diet in relation to overweight in the population of the Balearic Islands. A pilot study

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Neuropeptide Y (NPY) is an orexigenic neuropeptide that plays an important role in energy homeostasis and variants in NPY gene have been associated with metabolic syndrome (MS) and cardiovascular diseases (CVD). On the other hand, Mediterranean diet (MD) constitutes an example of a diet that could potentiate healthier outcomes and therefore, could modulate the genetic influence of NPY.

The aim of this study has been to assess the potential interaction between the presence of genetic variants of rs16147 (located in NPY promoter) and the degree of adherence to MD in relation with obesity.

Representative individuals living in Mallorca and over 18 years old were recruited (N=108). Anthropometric (height, weight, body fat percentage (BF%) and waist circumference (waistC)), dietary and genetic data were obtained. DNA was extracted from saliva and genotyped by q-PCR for rs16147. Adherence to MD was evaluated by the MEDAS Index (a 14-point validated questionnaire). Scores bigger than eight –population's mean - was considered "high adherence to the MD" (H-MD), whereas equal or less than eight was considered "low adherence to the MD" (L-MD).

Subjects L-MD that were carriers of the majority allele in homocygosis (AA) shown a significantly higher BMI, BF% and waistC compared with L-MD subjects having the minority allele (G). However, a better anthropometric profile was observed among carriers of alleles AA with high adherence to MD. Despite the relative low number of subjects, a significant interaction between the adherence to MD and this genetic variant in NPY gene has been observed. Particularly, AA carriers shown features associated with obesity when they had a low adherence of MD, whereas a leaner phenotype was found when adherence to MD was high. These results outline the relevance of incorporate the characterization of genetic baggage in the fight against obesity and to guide nutritional interventions.

Acknowledgments: S.G. is supported by a PhD fellowship by the "Conselleria d'Educació, Cultura i Universitats, Govern de les Illes Balears", as part of a program cofinanced by the European Social Fund.

## P-42 *LINE-1* and *SERPINE1* methylation levels in PBMCs as early biomarkers of weight loss, and relationship of *LINE-1* methylation with the dietary antioxidant capacity

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The epigenetic marks, and specially DNA methylation, have arisen as new tools to personalize the treatment of obesity and its comorbidities. The objectives of the current investigation were to study the changes in global DNA methylation levels (*LINE-1*) and in inflammatory genes (*SERPINE1, TNF* and *IL6*) before and after an 8-week dietary intervention, to identify predictive biomarkers of response to a hypocaloric diet, and to better understand the influence of some nutrients (particularly antioxidants) over the epigenome.

DNA methylation was assessed in peripheral blood mononuclear cells (PBMCs) from 96 obese volunteers of the RESMENA project, using a methylation-sensitive high resolution melting (MS-HRM) approach after bisulphite modification.

BMI values decreased after the energy-restricted program (from 35.8 kg/m$^2$ to 33.4 kg/m$^2$, p-value<0.001). The treatment increased the methylation levels of *SERPINE1* and *IL6*, but did not change the methylation levels of *LINE-1* and *TNF*.

Baseline *LINE-1* and *SERPINE1* DNA methylation levels were significantly higher in high responders (>8% of weight loss) as compared to low responders (<8%). Both, *LINE-1* and *SERPINE1* baseline DNA methylation levels significantly correlated with the response to the treatment, in the form of BMI, body weight, waist circumference and total fat mass.

Also, *LINE-1* baseline methylation levels correlated positively with baseline dietary total antioxidant capacity (TAC) as calculated by Carlsen et al. (2010). By using a linear regression model we observed that total fat mass and TAC explained about 22% of the variation of the baseline DNA methylation of *LINE-1.*

In summary, *SERPINE1* and *IL6* methylation levels in PBMCs are affected by the weight-loss treatment. *LINE-1* and *SERPINE1* baseline methylation levels in PBMCs might be used as predictive tools for weight loss achievement. Finally, *LINE-1* methylation levels seems to be related with the dietary total antioxidant capacity.

Acknowledgements: This work was supported by CIBERobn, Nutrigenio project (ref. AGL2013-45554-R), Línea Especial “Nutrition, Obesity and Health” (University of Navarra LE/97) and Health Department of the Government of Navarra (48/2009).

## P-43 Alimentomica: a technological-based company in Nutrition

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Alimentomica, founded in 2011, is the first technological-background based SME spin-off of the University of Balearic Islands (UIB) (http://www.alimentomica.es). Its mission is to promote and enhance opportunities for research and technology transfer of new developments or innovative practices in the nutrition field to improve people's diet and health. In addition to the core team of senior scientific-UIB partners, activities are developed by highly qualified experts. Alimentomica hires two doctors through the Spanish Torres Quevedo Programme, which foments innovation in enterprises through doctoral training. In addition, it participates in the Master of Nutrigenomics and Personalised Nutrition of UIB, allowing practical training of students in their premises. From that cooperation programme, two Master students are at present staff members in Alimentomica.

The company has three main lines of activity:

a) Perinatal nutrition: Devoted to the development and launching of products that may contribute to the prevention of adult obesity through the improvement of diet, particularly at very early ages. The portfolio includes the design of nutritionally improved formula-milks by the addition of bioactive components, normally found in breast milk, but not in currently available formula-milks; as well as, nutritional strategies aiming to optimize the concentration of bioactive components in breast milk through improvement of maternal diet.

b) Consultancy in novel food and health claims arena: Providing expert assistance along the process of preparation and submission of novel food & Health Claims applications.

c) Personalized nutrigenetic counseling: This has been implemented through the development and launching of Metigentity, a nutrigenetic test based in SNPs potentially related to obesity. Metigentity integrates the genetic identity with the habitual dietary pattern of the individuals and the actual knowledge on gene-diet interaction. Scientific evidences together with raw individual data are integrated in a web-based platform contributing to the design of patient's' personalized dietary recommendations by practitioners, nutritionists and medical doctors.

Acknowledgements: JM and MP are supported by Torres Quevedo contracts by the "Ministerio de Economía y Competitividad", as part of a program cofinanced by the European Social Fund and the involved company (Alimentomica).

## P-44 Effects of high versus low intake of bioactive food compounds on biomarkers of adipose tissue health, obesity- and metabolic syndrome-associated low-grade inflammation, dyslipidemia and disturbed glucose metabolism: cross-sectional data of the BIOCLAIMS cohort

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The aim of this study was to investigate the effects of high versus low intake of bioactive food compounds on established and new biomarkers. Study subjects of the highest (Q5) and lowest quintiles (Q1) of consumption of bioactive compounds have been identified in 1310 subjects of the BIOCLAIMS cohort (606 M, 704 F, age 18-85 years), which has been established by Karl-Franzens University and Medical University of Graz, Austria. Dietary intake was estimated from consumption frequencies in the preceding 12-month period, using a 240-item food frequency questionnaire (FFQ) with picture-supported average portion sizes.

Biomarkers of nutritional exposure (vitamins A, C, E, D, B12, folic acid, $\alpha$- and $\beta$-carotene, lycopene, $\beta$-cryptoxanthin, lutein/zeaxanthin, zinc), adipose tissue (leptin, adiponectin), vascular (homocysteine, HCY; asymmetric dimethylarginine, ADMA; intima-media thickness, IMT), inflammatory (C-reactive protein, CRP; interleukin-6, IL-6; sICAM-1, sVCAM-1, e-selectin) and oxidative stress status (human mercaptalbumin, HMA; malondialdehyde, MDA; total peroxides, POX) were determined. Q1 and Q5 were compared using Mann-Whitney U test, $P < 0.01$ was considered as significant difference.

Plasma levels were significantly related to intake of carotenoids, folate, vitamin C (all $P < 0.001$) and B12 ($P = 0.007$). Lower levels of plasma biomarkers were found for higher intakes (Q1>Q5) of *vitamin B12* for adiponectin ($P < 0.001$); *retinol* ($P = 0.002$) for leptin/adiponectin ratios; *carotenes, vitamin E, folate, zinc* (all $P < 0.001$) and *vitamin K* ($P = 0.001$) for HCY; *vitamin B12* and *zinc* for ADMA (all $P < 0.001$); *carotenoids, folate* (all $P < 0.001$) and *vitamin E* ($P = 0.001$) for HOMA index; *carotenoids* for CRP ($P = 0.007$) and IL-6 ($P = 0.009$); and *fiber* for cholesterol ($P < 0.001$). In contrast, higher levels were found for higher intakes (Q5 > Q1) of *carotenoids* ($P < 0.001$) and *retinol* ($P = 0.002$) for adiponectin ($P < 0.001$); and of *vitamin K* ($P = 0.002$) and *fiber* ($P < 0.001$) for MDA.

These results suggest that the biomarkers used in this study are sensitive to different intake levels of bioactive food compounds and thus could be useful for health claim support.

Acknowledgements: The research leading to these results has received funding from the European Union's 7th Framework Programme FP7 2007-2013 under grant agreement nº 244995 (BIOCLAIMS Project) and the Federal Ministry of Science, Research and Economy of Austria.

## P-45 Effects of high versus low intake of n-3 polyunsaturated fatty acids on biomarkers of adipose tissue health, obesity- and metabolic syndrome-associated low-grade inflammation, dyslipidemia and disturbed glucose metabolism: cross-sectional data of the BIOCLAIMS cohort

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The aim of this study was to investigate the effects of high versus low intake of fatty acids (FA) on established and new biomarkers. Study subjects of the highest (Q5) and lowest quintiles (Q1) of FA consumption have been identified in 1310 subjects of the BIOCLAIMS cohort (606 M, 704 F, age 18-85 years), established by the Karl-Franzens University and Medical University of Graz, Austria. Dietary intake was estimated from consumption frequencies in the preceding 12-month period, using a 240-item food frequency questionnaire (FFQ) with picture-supported average portion sizes.

Plasma concentrations of individual FA were measured and n-3 (including eicosapentaenoic acid, EPA; and docosahexaenoic acid, DHA), n-6 FA and other FA groups were calculated as biomarkers of nutritional exposure. Adipose tissue (leptin, adiponectin), vascular (homocysteine, HCY; asymmetric dimethylarginine, ADMA; intima-media thickness, IMT), inflammatory (C-reactive protein, CRP; interleukin-6, IL-6; sICAM-1, sVCAM-1, e-selectin) and oxidative stress status (human mercaptalbumin, HMA; malondialdehyde, MDA; total peroxides, POX) were determined.

Plasma levels were significantly related to intake of DHA, linoleic, linolenic and arachidonic acid as well as total n-3 and n-6 FA ($P < 0.001$ or $P = 0.001$) and EPA ($P = 0.018$). Lower levels of plasma biomarkers were found for higher intakes (Q1 > Q5) of *DHA* ($P < 0.001$) and *EPA* ($P = 0.002$) for leptin; *arachidonic acid* ($P < 0.001$) for adiponectin; *DHA* ($P = 0.004$), *EPA* ($P = 0.009$), and *total n-3 FA* ($P = 0.007$) for leptin/adiponectin ratios; *total n-3 FA, linolenic acid, DHA* (all $P < 0.001$), EPA ($P = 0.001$), MUFA (P = 0.006) and oleic acid (P = 0.002) for HCY; *DHA* for ADMA ($P < 0.001$); and n-3 FA ($P < 0.001$) for HOMA index. In contrast, higher levels were found for higher intakes (Q5 > Q1) of *linolenic acid* ($P < 0.001$) and *n-3 FA* ($P = 0.003$) for adiponectin ($P < 0.001$), *DHA* ($P < 0.001$) and *arachidonic acid* ($P < 0.002$) for homo-arginine ($P < 0.001$); *n-3 FA* ($P = 0.005$) and *linolenic acid* ($P = 0.008$) for MDA; and *oleic acid* ($P = 0.009$) for HMA.

These results demonstrate (a) that the FFQ used in this study was reliable to assess dietary FA intake, (b) that plasma levels are associated with intake, and c) that the biomarkers that are used in the study are sensitive to different fatty acid intake levels and thus could be useful for quantifying effects of n-3 fatty acid supplementation and related health claim support.

Acknowledgements: The research leading to these results has received funding from the European Union's 7th Framework Programme FP7 2007-2013 under grant agreement nº 244995 (BIOCLAIMS Project) and the Federal Ministry of Science, Research and Economy of Austria.

## P-46 Effects of a Mediterranean-type diet on biomarkers of vascular and metabolic health status in the BIOCLAIMS cohort

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While dietary intake studies frequently focus on quantification of intake of individual dietary components, dietary intake patterns might be even more relevant in terms of health effects. The aim of this study was to investigate the effects of adherence to a Mediterranean-type diet on biomarkers of vascular and metabolic health status. Adherence to this diet has been determined from a 240-item food frequency questionnaire (FFQ) with picture-supported average portion sizes, using the alternate Mediterranean Diet Score (aMED score, Fung et al., AJCN 2005;82:163-73).

A total of 1310 study subjects (606 males and 704 females, aged 51.3±16.1 years) were assigned gender- and total energy intake-adjusted aMED scores of 0-9. Biomarkers of vascular (homocysteine, HCY; asymmetric dimethylarginie, ADMA; intima-media thickness, IMT; ICAM and VCAM) and metabolic health (leptin, adiponectin, HOMA index, total and LDL cholesterol, malondialdehyde, MDA) were determined along with anthropometric indices (body mass index, BMI; waist and hip circumference; subcutaneaous adipose tissue, SAT, by Lipometer®; total body fat, BF, by bioelectrical impedance, BIA). Study subjects with aMED score of 0-2 (low aMED, n=242) were compared with those with aMED score of 7-9 (high aMED, n=177) for biomarkers of vascular and metabolic health and anthropometric indices. Mann-Whitney U tests were used for the comparisons, $P < 0.01$ was considered significant.

Body mass index (P<0.001), waist (P<0.001), hip (P=0.001), SAT (P<0.001, BF (P=0.002) were significantly higher in subjects with low aMED scores, as well as leptin and leptin:adiponectin ratios (both $P < 0.001$), CRP (P=0.005), myeloperoxidase ($P < 0.001$), HCY ($P < 0.001$), ) and HOMA index ($P < 0.001$), while MDA (*P<0.001)* was higher compared to subjects with high aMED scores.

These results demonstrate that (a) the aMED score applied in a population with relatively low adherence to a Mediterranean diet is able to demonstrate gaps for the improvement of nutritional status and (b) that the biomarkers that were used in our study are sensitive to detect significant effects of dietary adherence patterns and therefore could be used for health claim support.

Acknowledgements: The research leading to these results has received funding from the European Union's 7th Framework Programme FP7 2007-2013 under grant agreement nº 244995 (BIOCLAIMS Project) and the Federal Ministry of Science, Research and Economy of Austria.

## P-47 Effects of a Cafeteria-type diet on biomarkers of vascular and metabolic health status in the BIOCLAIMS cohort

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Cafeteria diet is used for inducing obesity and metabolic syndrome in animal experiments (Chen et al., PLOSone 2014;9:e102213). In relation to human diet Cafeteria diet is characterized by readily accessible, energy dense, palatable foods rich in fat, sugar and carbohydrates, which cause excessive food intake and rapid weight gain.

The aim of this study was to investigate the effects of adherence to a Cafeteria-type diet on biomarkers of vascular and metabolic health status. Adherence to this diet has been determined using a 240-item food frequency questionnaire (FFQ) with picture-supported average portion sizes. A Cafeteria diet (CafeD) score has been developed, consisting of 5 components (refined grain products; animal fats; ready-for-use products; pastries and salty snacks and sweetened soft drinks and sugar), with a CafeD score of 5 assigned for highest and score of 0 for absence of intake.

A total of 1310 study subjects (606 males and 704 females, aged 51.3±16.1 years) were assigned gender- and total energy intake-adjusted CafeD scores of 0-5. Biomarkers of vascular (intima-media thickness, flow-mediated dilatation, homocysteine, asymmetric and symmetric dimethylarginine, ADMA, SDMA; vascular adhesion molecules) and metabolic health (leptin; adiponectin; HOMA index; total and LDL cholesterol), inflammation (CRP, interleukin-6 myeloperoxidase, MPO), oxidative stress (human mercaptalbumin, HMA; malondialdehyde, MDA) were determined along with anthropometric indices (body mass index, waist and hip circumference, subcutaneous adipose tissue distribution, SAT, from Lipometer, body fat mass from bioelectrical impedance. Study subjects with CafeD scores of 0-1 (low CafeD, n=283) were compared with those with CafeD of 4-5 (high CafeD, n=263) for biomarkers of vascular and metabolic health, along with anthropometric indices. One-way ANOVA with age as a covariate was used for comparison, because subject with low CafeD scores were significantly older than those with high scores. $P < 0.01$ was considered significant. SAT mass (P<0.001), MPO (P=0.004) and HMA (P=0.04) were higher in the high CafeD, while MDA ($P < 0.001$) was higher in the low CafeD.

These results demonstrate that a high CafeD score is not associated with impaired vascular and glucose metabolism.

Acknowledgements: The research leading to these results has received funding from the European Union's 7th Framework Programme FP7 2007-2013 under grant agreement nº 244995 (BIOCLAIMS Project) and the Federal Ministry of Science, Research and Economy of Austria.

## P-48 Dicarbonyl stress in adipose tissue and liver of mice fed an obesogenic diet

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To assess the presence of dicarbonyl stress in white adipose tissue (WAT) and liver of mice on an obesogenic high-fat diet (HFD) and the effect of ectopic expression of mitochondrial uncoupling protein-1 (UCP1) in WAT.

Dicarbonyl stress, the abnormal accumulation of dicarbonyl metabolites such as methylglyoxal (MG) leading to cell and tissue dysfunction, has recently emerged as a possible contributory factor to obesity. MG is metabolised mainly by glyoxalase 1 (Glo1).

At 3 months of age, wild-type (WT) male C57BL/6J mice and their transgenic littermates expressing UCP1 gene under the adipocyte lipid-binding protein gene promoter (aP2-*Ucp1* mice) were randomised and fed control chow (3.4% fat) or HFD (*ca.* 35% fat) for 10 weeks. Mice were sacrificed following overnight fasting. Epididymal WAT and liver were used for experiments. MG was measured by LC-MS/MS and glyoxalase 1 (Glo1) activity by spectrophotometric assay.

MG content of WAT (pmol/mg wet weight) was increased by HFD in WT mice, with respect to control chow (control, 1.47 ± 0.65 vs HFD, 2.91 ± 0.98, +98%, $P<0.01$; n = 7 – 8). A similar increase was not found in aP2-*Ucp1* mice (control, 1.87 ± 0.73 vs HFD, 2.51 ± 0.64; n = 5 - 6). Increased MG was associated with decreased Glo1 activity in WAT of WT mice on a HFD, which was not found in aP2-*Ucp1* mice. Glo1 activity (U/mg protein): WT, HFD 13.7 ± 4.9 (-28%, $P<0.05$) vs control 19.0 ± 2.9; aP2-*Ucp1*, HFD 18.3 ± 6.4 vs control 20.7 ± 2.1 (n = 5 – 8). MG content was also increased in liver of WT mice on HFD (+84%, $P<0.001$) and not in aP2-*Ucp1* mice. This was not associated with decreased hepatic Glo1 activity but rather is likely linked to increased flux of MG formation.

Mice on an obesogenic diet suffer dicarbonyl stress in WAT and liver, linked to down regulation of Glo1 activity in WAT. Dicarbonyl stress and the obesity phenotype were prevented in aP2-*Ucp1* mice.

Acknowledgements: We thank the European Union Framework Programme-7 for support for the BIOCLAIMS research programme, grant agreement no. 244995 and grant K/ZDS/002442.

## P-49 Decreased methylglyoxal-modified protein damage and proteome re-structuring in the HSA-UCP1 mouse model of healthy ageing

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Ectopic expression of uncoupling protein 1 (UCP1) under a human skeletal muscle actin promoter in skeletal muscle of C57BL/6 mice increased lifespan. We investigated the effect on endogenous protein damage of skeletal muscle and plasma in young (12 wk) and old (70 wk) wild-type (WT) and transgenic (Tg) mice. Protein glycation, oxidation and nitration adducts were quantified by stable isotopic dilution analysis LC-MS/MS.

Glycoxidation adduct $N_\varepsilon$-carboxymethyl-lysine (CML)/$N_\varepsilon$-fructosyl-lysine (FL) ratio, a marker of oxidative stress, was increased in muscle and plasma of old WT type mice. This was prevented in skeletal muscle but not in plasma protein of Tg mice. CML/FL ratio in muscle: young – WT, 0.90 [0.51 – 1.09] vs Tg, 0.44 [0.40 – 0.47]($P<0.05$); old – WT - 1.08 [0.79 - 1.21] vs Tg, 0.61 [0.48 - 0.64]($P<0.01$). FL content, a marker of glycaemia, was increased in skeletal muscle of young but not old Tg mice, 0.201 ± 0.074 vs 0.117 ± 0.025 mmol/mol lys ($P<0.01$), indicating increased glucose uptake in muscle of young Tg mice. Methylglyoxal (MG)-derived glycation adduct MG-H1, a marker of dicarbonyl stress and mitochondrial dysfunction, was decreased in skeletal muscle of old Tg mice, with respect to WT controls contents: 0.268 ± 0.025 versus 0.370 ± 0.052 mmol/mol arg ($P<0.001$). MG is metabolised by glutathione (GSH) –dependent glyoxalase 1 (Glo1). Skeletal muscle contents of MG, GSH and Glo1 activity were unchanged in Tg mice, suggesting that re-structuring of fibres resistant to MG modification rather than decreased MG exposure was involved. High resolution quantitative proteomics of muscle of old mice identified and quantified 492 proteins common to WT and Tg mice, 20 proteins (other than UCP1) unique to Tg mice and 7 proteins modified by MG-H1. The latter included: myosin regulatory light chain-2, GAPDH, creatine kinase b-type and β-actin. Decreased dicarbonyl stress and related protein damage may contribute to the healthy ageing phenotype.

Acknowledgements: We thank the European Union Framework Programme-7 for support for the BIOCLAIMS research programme, grant agreement no. 244995 and grant K/ZDS/002442.

## P-50 Protein glycation, oxidation and nitration in clinical obesity and effect of EPA/DHA supplementation - BIOCLAIMS study

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To examine levels of protein glycation, oxidation and nitration markers in obese and non-obese subjects as potential markers of metabolic homeostasis and assess the effect of *n*−3 polyunsaturated fatty acid (PUFA) supplementation.

Non-obese subjects on isocaloric diet (BMI 27.8 ± 2.0 kg/m$^2$, n = 36), obese subjects on isocaloric diet (BMI 34.3 ± 3.5 kg/m$^2$, n = 58), and obese subjects on a low caloric diet (BMI 34.8 ± 3.5 kg/m$^2$, n = 52). Isocaloric diet was 2300 – 2400 kcal/day and low caloric diet 1200 kcal/day (women) and 1500 kcal/day men (men).

Supplementation was randomized to PUFA EPAX1050TG (Norway) – 1.8 g docosahexaenoic acid/eicosapentaenoic acid (5:1) + 12 mg of vitamin E per day for 3 months or placebo (corn oil + 12 mg vitamin E). Sampling was fasted state plasma and urine (morning second void). Protein glycation, oxidation and nitration adducts were measured in plasma protein (adduct residues) and urine (free adducts) by LC-MS/MS.

Plasma protein glycation, oxidation and nitration were unchanged in isocaloric diet obese versus non-obese subjects. In obese subjects, the dicarbonyl stress marker - methylglyoxal-derived adduct MG-H1/glucose-derived $N_\varepsilon$-fructosyl-lysine (FL) ratio - was decreased on a low calorie diet; 0.17 ± 0.12 vs 0.13 ± 0.08 ($P<0.05$). In obesity, compared to non-obese isocaloric controls, there was dysglycaemia in tissues – indicated by increased urinary excretion of FL (+43%) and 3DG-H (+57%), increased oxidative damage – increased urinary excretion of N-formylkynurenine (+226%), and dicarbonyl stress - increased urinary excretion of MG-H1 (+37%). In obesity, PUFA supplementation improved dysglycaemia (urinary FL and pentosidine, -28% and -31% respectively), and oxidative damage (urinary methionine sulfoxide and 3-nitrotyrosine, -12% and -21% respectively).

Dysglycaemia, oxidative damage and dicarbonyl stress were increased in clinical obesity. A low calorie diet improved this. PUFA supplementation improved dysglycaemia and oxidative damage but not dicarbonyl stress.

Acknowledgements: We thank the European Union Framework Programme-7 for support for the BIOCLAIMS research programme, grant agreement no. 244995 and grant K/ZDS/002442.

## P-51 Antioxidant response element cytoprotective response in aortic endothelial cells coordinated by transcription factor Nrf2 is regulated through frequency-modulated translocational oscillations

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To examine how Nrf2 signals cell stress status and regulates transcription to maintain homeostasis for good vascular health.

Nrf2 regulates the cellular expression of a battery of protective genes countering oxidative stress, environment toxic insults, lipid peroxidation, macromolecular damage, metabolic dysfunction and cell senescence. It binds to regulatory anti-oxidant response elements (AREs).

We used live cell time-lapse video microscopy, ARE transcriptional reporter assays, mRNA transcriptional profiling and immunoblotting to study Nrf2-coordinated ARE-transcriptional response in human aortic endothelial cells and HMEC-1 cell line.

We found that Nrf2 is activated in cells without change in total cellular Nrf2 protein concentration. Rather, in live cell microscopy we observed that Nrf2 undergoes autonomous translocational frequency-modulated oscillations between cytoplasm and nucleus. Oscillations occurred in quiescence and when cells were stimulated at physiological levels of activators, they decrease in period and amplitude and then evoke a cytoprotective transcriptional response. Nrf2 was inactivated in the nucleus and reactivated on return to the cytoplasm. Increased frequency of Nrf2 return to the cytoplasm with increased reactivation or refresh-rate under stress conditions activated the transcriptional response mediating cytoprotective effects. Post-translational phosphorylations and acetylation of Nrf2 control and coordinate this process.

Frequency modulated translocational oscillations of Nrf2 mediate the ARE-linked cytoprotective transcriptional response**.** Nrf2 activator design for prevention of vascular disease has, to date, employed a strategy of maximizing residence time of Nrf2 in the nucleus which may rather have selected for suboptimal if not poor inducers of the ARE-linked transcriptional response. A more effective approach may be to ‘‘tune’’ Nrf2 translocational oscillation frequency to maximize functionally active Nrf2 in the cell nucleus. Moreover, our studies indicate there are multiple receptors for activation of Nrf2 and provide a rational basis for dietary bioactive co-formulation for improved functional food development to maintain good metabolic and vascular health.

Acknowledgements: We thank the Biosciences and Biotechnology Research Council (BBSRC), U.K. for research funding - Diet and Health Research Industry Club (DRINC) project grant no 07/84, and the European Union Framework Programme-7 for support for the BIOCLAIMS research programme, grant agreement no. 244995 and grant K/ZDS/002442.

## P-52 Validation of new biomarkers in the BIOCLAIMS Integration study: Plasma acylcarnitines and amino acids, fatty acids, leptin/adiponectin, human mercaptalbumin and nuclear factor kB in study subjects with mildly impaired renal, vascular and metabolic health compared to "super-healthy" subjects

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*The aim of this study* was to identify and validate new biomarkers in an integrated approach in samples of the BIOCLAIMS cohort consisting of 1310 study participants, 607 M, 704 F, aged 18-85 y, which were available for selection of 50 study subjects each into 4 contrasting groups:

*Group 1*, *impaired renal health* (glomerular filtration rate eGFR 30-60 ml/min/1.73 $m^2$), *Group 2*, *impaired vascular health* (intima-media thickness left and right >75$^{th}$ percentile, *Group 3, impaired metabolic health* (HOMA index >2.5 and HbA1c 38.8-44 mmol/mol), and *Group 4*, *"super" healthy subjects as a human control model* (clinical chemistry variables within normal range ±10%, IMT not >75$^{th}$ percentile at both sides, and not taking medications). Care was taken to avoid overlaps between the groups, while matching for age was not possible, given the evolution of impaired health as a function of age (age group 1 > groups 2 and 4 and group 3 > group 4).

*Plasma metabolomics biomarkers* (28 acylcarnitines, 17 amino acids and 21 plasma fatty acids) showed significant ($P$ < 0.001) differences between groups for serine, threonine, histidine (4 > 1) and glycine+lysine, glutamine and carnitine (1 > 4); all plasma acylcarnitines differed significantly ($P$ < 0.001; 1 >2, 3, 4) except C22-6 and C6DC+C7OH (4 > 1). Fatty acid profiles also differed significantly, such as C22:4n-6 ($P$ < 0.001; 1 > 2, 3, 4), C22:5n-3 ($P$ = 0.006; 1 > 3, 4), C22:6n3 ($P$ = 0.002; 1 < 2, 4). *Leptin:adiponectin ratios* were higher ($P$ < 0.001; 3 > 2, 4 and 1 > 4) in the metabolic and renal impairment groups. *HMA:HNA1 ratios* differed significantly ($P$ < 0.001; 2 ,3, 4 > 1, 4 > 3) in the presence of significant differences ($P$ < 0.001) in antioxidant status (ascorbate, 1 < 2, 4, 2 > 3, 4 > 3; $\alpha$-tocopherol, 1, 3 > 4). Two additional biomarkers (activation of the redox-sensitive transcription factor NF-κB in PBMC, presented in another abstract of our group and fibroblast growth factor FGF21,will also be included in upcoming validation.

These results demonstrate significant differences for promising new biomarkers of health between subjects with impaired health and the human super healthy model that could be used for health claim support. Further validation and modelling is on-going.

Acknowledgements: The research leading to these results has received funding from the European Union's 7$^{th}$ Framework Programme FP7 2007-2013 under grant agreement nº 244995 (BIOCLAIMS Project) and the Federal Ministry of Science, Research and Economy of Austria.

## P-53 Activation of p50 and p65-containing nuclear factor kB dimers in PBMC in the BIOCLAIMS cohort

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NF-κB signaling plays a role in the regulation of different genes encoding for central regulators of inflammatory processes. Due to its redox sensitivity, NF-κB is an important interface between oxidative stress and inflammation. Activation of nuclear factor NF-κB subunits *p65* and *p50* in PBMC has been tested in studies of the BIOCLAIMS cohort, including a cross-sectional study in 629 study subjects (369 M, 260 F), a menstrual cycle study in 28 women, and the BIOCLAIMS Integration (BIG) study in 4 contrasting groups with a total of 173 study subjects (93 M, 80 F).

Highly specific NF-kB-DNA binding activity in whole cell extracts of PBMC has been determined, using the TransAM (Active Motif) assay. All study subjects were genotyped for the NF-kB1 single nucleotide polymorphism rs28362491; genotype frequencies were 36.4 % for INS/INS, 49.4 % for INS/DEL, and 14.2 % for DEL/DEL. Given that effects of drugs (antihypertensives, statins, oral antidiabetics) on NF-kB activation have been reported, intake of drugs has been taken into account. Non-users of drugs were assigned to *group 1(*n=234); users of the above described drugs to *group 2 (*n=264), and users of other drugs to group 3 (n=131). *P50* (OD) differed significantly (P=0.001) among groups, with group 1 (0.765±0.392) > group 2 (0.660±0.271) and group 3 (0.633±0.241). *P65* was significantly (P<0.001) different between group 1 (1.031±0.327) > group 3 (0.909±0.306) > group 2 (0.794±0.307). Within the non-users, stratification revealed significant (P=0.006) differences for p50: INS/INS (0.824± 0.395) > DEL/DEL (0.819± 0.409) >INS/DEL (0.698±0.375), while such differences were not found in the other groups nor for p65 in any group. In subjects participating in the BIG study, both p50 (P=0.007) and p65 (<0.001) showed significant differences between the 4 contrasting groups: *p50* levels in healthy subjects not taking medications were 0.796±0.392, which confirmed the values of group 1 shown above, in contrast to 0.575±0.183 in patients with impaired renal function (P<0.05), 0.730±0.346 in subjects with impaired vascular and 0.885±0.558 with impaired metabolic health (P<0.05). *P65* in healthy subjects was 1.032±0.357; i.e. similar to group 1 shown above, and significantly higher than in patients with impaired renal function (0.821±0.292). In the menstrual cycle study, *p65* showed significant (P=0.014) changes across the cycle with highest levels in the early follicular phase

(1.040±0.270) compared to 0.898±0.168 in the mid luteal phase, while *p50* levels did not change significantly (0.786±0195 at early follicular phase). Regression analysis in non-users (group 1) revealed a positive association of *p65* with plasma total PUFA (P=0.003) and a negative association with SFA (P<0.001) and CRP (P=0.035).

These most comprehensive and consistent data on NF-kB activation available so far suggest that NF-kB is a promising new biomarker of health as compared to impaired health status. Further validation in the BIOCLAIMS cohort is on-going.

Acknowledgements: Supported by European Union's 7th Framework Programme FP7 2007-2013 under grant agreement nº 244995 (BIOCLAIMS Project) and the Federal Ministry of Science, Research and Economy of Austria.

## P-54 Effects of gender, menopause, hormonal contraceptives and breastfeeding in infancy on biomarkers of adipose tissue, vascular and metabolic health in the BIOCLAIMS cohort

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The aim of this study was to reveal whether or not associations between biomarkers and health endpoints are affected by age and gender and other potential independent variables such as menopause, intake of female sexual hormones and breastfeeding in infancy.

A total of 1310 study subjects of the BIOCLAIMS cohort, including 606 men and 704 women, aged 18-85 (mean±SD, 51,3±16.1 years), were included in the analysis. A total of 336 females were in menopause and 137 women used hormonal contraceptives. Information on breastfeeding in infancy was available for 609 subjects.

Focus was directed on biomarkers of inflammation (C-reactive protein, CRP; interleukin-6, IL-6; myeloperoxidase, MPO); adipose tissue (leptin; adiponectin; leptin:adiponectin ratio) and vascular health (asymmetric dimethylarginine, ADMA; homocysteine, HCY; vascular adhesion molecules, ICAM and VCAM) along with vascular health endpoints (intima media thickness, IMT; flow-mediated dilatation, FMD; blood pressure), anthropometric indices (body mass index, BMI; subcutaneous adipose tissue, SAT mass by Lipometer®; total body fat, BF, by bioelectrical impedance; phase angle) and dietary exposure (plasma fatty acid profile, including n-3 and n-6 PUFA, MUFA and individual FA; vitamin A, C, E, D status).

Females showed higher BF and SAT mass, leptin, adiponectin and leptin:adiponectin ratios, total and HDL cholesterol, as well as higher plasma n-3 and n-6 PUFA (all $P < 0.001$), in contrast to higher BMI, waist circumference, phase angle, HCY, MPO, IMT, MUFA, retinol and $\alpha$-tocopherol:cholesterol (all $P < 0.001$) and IL-6 ($P = 0.003$) in males. Biomarkers of dietary exposure showed higher plasma vitamin C, n-3 and n-6 PUFA in females and higher plasma MUFA in males (all $P < 0.001$). Menopause had a significant effect ($P < 0.001$ or $P < 0.01$) on all variables except IL-6 and CRP. Intake of hormonal contraceptives was associated with lower ADMA ($P < 0.001$) and higher hArg ($P = 0.02$) and HOMA index ($P = 0.024$). Subjects breastfed in infancy (n=533) showed lower SAT mass ($P = 0.002$), BF ($P = 0.023$), and hip circumference ($P = 0.03$).

These results demonstrate that gender and menopause have significant effect on the biomarkers included in the analysis and need to be taken into account for validation biomarkers for health claim support.

Acknowledgements: The research leading to these results has received funding from the European Union's 7th Framework Programme FP7 2007-2013 under grant agreement nº 244995 (BIOCLAIMS Project) and the Federal Ministry of Science, Research and Economy of Austria.

## P-55 Leptin supplementation in suckling rats restores altered white and brown adipose tissue function caused by maternal calorie restriction during gestation

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Maternal calorie restriction during gestation programs offspring for later overweight and metabolic disturbances. Early alterations in white and brown adipose tissues (WAT and BAT, respectively) have been described to be determinant for these later disturbances. The shortcomings translate in a dysfunction of WAT and a lower thermogenic capacity of BAT, both related to a decrease in adrenergic innervation. The anorexigenic hormone leptin has been identified as a factor present in breast milk, and hence ingested during the suckling period, that may help the offspring to prevent obesity and other metabolic alterations later in life.

We aimed to investigate whether oral supplementation of neonate rats with physiological doses of leptin during the suckling period may ameliorate the adverse developmental malprogramming effects exerted in offspring WAT and BAT sympathetic innervation and function due to maternal undernutrition during gestation. Three groups of male rats were studied after weaning: the offspring of *ad libitum* fed dams (controls), the offspring of 20% calorie restricted dams during the first part of pregnancy (CR), and CR rats supplemented with physiological doses of leptin throughout lactation (CR-Leptin). Leptin treatment was able to restore the decreased sympathetic innervation showed by CR animals in WAT, which was accompanied by partial normalization of the underexpressed genes related to lypolisis and fatty acid oxidation. In BAT, CR-Leptin animals also showed a partial recovery of uncoupling protein 1 (UCP1) levels, which were decreased in CR animals, and an improvement in the thermogenic response to cold exposure, which was also impaired due to maternal calorie restriction. These results support the relevance of the intake of leptin during the suckling period, a specific compound of maternal milk, which may be considered as a strategy to treat and/or prevent the adverse effects of undernutrition during gestation, and hence making this metabolic malprogramming reversible to some extent.

Acknowledgements: Spanish Government (grant AGL2012-33692), the European Union's Seventh Framework Programme FP7 2007-2013 under grant agreement n. 244995 (BIOCLAIMS Project), and the Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición, CIBERobn. N. S. is granted with a PhD fellowship entitled "beca para la formación de personal investigador", co-funded by Regional Government (Conselleria d'Educació, Cultura i Universitats, CAIB) and European Social Fund (FSE).

## P-56 Genetic polymorphisms in the BIOCLAIMS cohort

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Genotype variants are believed to differentially modulate the inflammatory response. Relevant single nucleotide polymorphisms (SNPs) are found in genes encoding for pro- and anti-inflammatory cytokines, nuclear receptors and nuclear transcription factors. For testing in the BIOCLAIMS cohort, the following 21 candidate SNPs have been selected, based on involvement of gene product in regulation of vascular and metabolic health and food preferences; minor allele frequencies (MAF) of ≥5%, association of SNP with gene expression or activity of the gene product: *(1)* rs1800587 (C-889T) in the interleukin (IL)-1α gene, *(2)* rs16944 in the IL-1β gene, *(3)* rs1800796 and *(4)* rs1800795 in the IL-6 gene, *(5)* rs1800871, *(6)* rs1800896 and *(7)* rs1800872 in the IL-10 gene, *(8)* rs361525 and *(9)* rs1800629 in the TNF-α gene, *(10)* rs909253 in the lymphotoxin (LT)-α gene, *(11)* rs1801282 in the PPAR-γ gene and *(12)* rs28362491, a -94 ATTG insertion/deletion SNP in the NFκB1 gene. SNPs that influence sensory perception included *(13)* rs713598, *(14)* rs1726866 and *(15)* rs10246939 in the TAS2R38 gene, which encodes for a G protein-coupled receptor that controls bitter taste perception. SNPs that modulate the antioxidant enzymes glutathione S-transferase (GST) and superoxide dismutase (SOD) *(16)* rs1138272 and *(17)* rs1695 are found in the GSTP1 gene and *(18)* rs4880 in the SOD2 gene. The NOS3 gene encodes for the endothelial nitric oxide synthase (eNOS) that generates NO in endothelial cells and regulates smooth muscle tone. *(19)* Rs1799983 is found in the NOS3 gene and modulates eNOS activity. *(20)* Rs1801133 and *(21)* rs1801131 are found in the MTHFR gene and influence the folic acid methyl cycle as a determinant of vascular disease.

A total of 1310 study subjects of the BIOCLAIMS cohort were genotyped for these SNPs. Genotypes were determined by fluorogenic 5-exonuclease assays (TaqMan™), primer and probe sets were designed and manufactured by Applied Biosystems (Life Tech). Endpoint fluorescence was measured in a POLARstar plate reader (BMG Labtech). For quality control, compliance with Hardy-Weinberg equilibrium (HWE) was tested and all SNPs were compliant with HWE. The MAF (%) and the HWE ($X^2$) in our study population were as follows: MAF and HWE of the SNP rs1800587 (IL-1 alpha) were 28.8 and 1.31, for rs16944 (IL-1β) 33.3 and 0.735, for rs1800796 (IL-6) 5.65 and 0.009, for rs1800795 (IL-6) 41.5 and 1.32, for rs1800871 (IL-10) 27.0 and 1.06, for rs1800896 (IL-10) 45.3 and 0.773, for rs1800872 (IL-10) 25.3 and 0.017, for rs361525 (TNF-α) 3.78 and 0.737, for rs1800629 (TNF-α) 15.2 and 1.19, for rs909253 (LT-α) 30.3 and 1.72, for

rs1801282 (PPAR-γ) 15.2 and 0.054, for rs28362491 (NFκB1) 37.9 and 0.408, for rs713598 (TAS2R38) 41.5 and 2.22, for rs1726866 (TAS2R38) 45.5 and 0.719, for rs10246939 (TAS2R38) 45.5 and 0.558, for rs1138272 (GSTP1) 10.7 and 0.575, for rs1695 (GSTP1) 35.2 and 2.07, for rs4880 (SOD2) 49.7 and 2.06, for rs1799983 (NOS3) 31.6 and 0.283, for rs1801133 (MTHFR) 34.0 and 0.186 and for rs1801131 (MTHFR) 32.9 and 0.412. Possible associations of SNPs with NF-κB activation in PBMC (presented in another abstract), inflammation, oxidative stress, vascular function, folate status and thresholds of sensory perception are under investigation.

Acknowledgements: The research leading to these results has received funding from the European Union's 7th Framework Programme FP7 2007-2013 under grant agreement nº 244995 (BIOCLAIMS Project) and the Federal Ministry of Science, Research and Economy of Austria.

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This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under agreement nº 244995

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