Reflections on Biomarkers & Lifestyle

Interaction Meeting

BIOCLAIMS-CIBEROBN

& V BIOCLAIMS Meeting

Palma de Mallorca, June 7-8th 2012

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Preface

The project "BIOmarkers of Robustness of Metabolic Homeostasis for Nutrigenomics-derived Health CLAIMS Made on Food" (BIOCLAIMS, Grant agreement no. 244995) is a collaborative, large-scale integrated research project funded by the European Commission through its 7 FP. The BIOCLAIMS project attempts to identify new "biomarkers" of the effects of food and food components on health, based on the new biological technologies, in particular, those of Nutrigenomics that may contribute with scientific bases for the reshaping of the European Legislation on "Health claims made on food" (EU Regulation 1924/2006). The scientific progress along the two running years of the progress has been considerable, particularly in those aspects covering methodological issues, analysis of animal models and assessment of potential biomarkers.

The Biomedical Network for Research in the Physiopathology of Obesity and Nutrition (CIBERobn) is a Spanish research consortium, supported by the Carlos III Health Institute (ISCIII), which involves outstanding Spanish research groups in the field. CIBERobn mission is to assist in the generation of knowledge that may allow a better understanding of the mechanisms that contribute to the development of obesity and to reduce its incidence and associated complications. Through its leadership in translational biomedical research, CIBERobn integrates basic, clinical, epidemiological and public health research, in combination with medical care and education in the field of obesity and nutrition. As a whole, CIBERobn constitutes a research center of reference able to provide R&D+i advisory services at companies and at food and health authorities of the country.

The points of convergence between both scientific networks are the starting point to get a higher degree of interconnectivity, synergy and dissemination of novel knowledge. Thus, the coordinator of the BIOCLAIMS project, who is also coordinator of the CIBERobn research program “New strategies and biomarkers in the prevention and treatment of obesity and food disorders”, proposed and has been encouraged to organize a BIOCLAIMS-CIBERobn interaction meeting, an event that will bring together people working in similar areas but from different points of views. We hope that this one-day meeting will allow discussing different approaches, strategies and tools against obesity, combining biomarkers discovery and lifestyle strategies to get healthier European citizens.

On behalf of the Organizing Committee, we welcome all of you to Palma de Mallorca and wish you an enjoyable and fruitful stay.

Andreu Palou
(BIOCLAIMS Coordinator)

Paula Oliver
(President of the Organizing Committee)

Palma de Mallorca, June 2012

Reflections on Biomarkers & Lifestyle

The identification of early biomarkers of susceptibility to disease (e.g. obesity) would allow relevant interventions with lifestyle and nutritional approaches before the disease is manifested and, therefore, may contribute to delay its onset or even better to refrain it. The Symposium on Biomarkers & Lifestyle: Interaction Meeting BIOCLAIMS-CIBERobn- joining BIOCLAIMS and CIBERobn scientists, has put on evidence novel insights concerning these two main aspects.

A number of contributions have presented proofs of a set of potential early biomarkers of obesity in animal models of genetic and dietary obesity. Early metabolic outputs representative of protection against obesity in adult life have also been characterized and biomarkers of unbalanced diets have been found in animal models. Transcriptomic analysis and microarrays give a non-targeted view of the metabolic pathways involved. In addition, targeted analysis of specific genes, transcriptional factors and other potential regulators contribute to the comprehension and fine tuning of the situation analyzing the effects in different tissues. The role of specific fatty acids has been outlined. Excess fatty acids may contribute to cellular stress but n-3 fatty acids may also improve the efficacy of medical treatment of obese and diabetic patients and a core of fatty acids may act as biomarker of susceptibility to obesity in adult life in a gender-specific animal model of obesity. A number of early indicators of susceptibility of obesity outline the potential role of sympathetic innervation indicators, which reflects early alteration in the regulatory circuitry of feeding behavior and could involve executive dysfunction in individuals with extreme weights. A subset of blood cells, PBMC are being consolidated as a cellular system that reflect the body situation at risk and constitute an easy source of biological material for sampling useful in the search of early biomarkers of disease. Bioactive compounds to counteract biomarkers of higher susceptibility of obesity and/or to sustain biomarkers of protection have also been presented.

Two main lifestyle interventions against obesity are changing dietary pattern and physical activity. A tool to assess dietary compliance to Mediterranean diet and its relevance in combination with genetic variants at risk has been presented. Some contributions are analyzing the impact of games as tools in obesity management particularly in children. Videogames can help to manage specific emotions in eating disorders and active games can contribute to promote and enhance physical activity. Maternal smoking affects the epigenome of low birth weight newborns which could be relevant in their future outcome.

Finally, these approaches, strategies and tools against obesity, result of the research of excellence carried out in European laboratories, may act as driving force to enhance competitiveness of food industry, contributing to improved diets and decreasing the risk of diseases in consumers/citizens.

A. Palou, F. Serra & P. Oliver

FINAL PROGRAMME

V BIOCLAIMS Meeting
&
Symposium on Biomarkers & Lifestyle:
Interaction Meeting BIOCLAIMS–CIBERobn

Thursday 7th & Friday 8th June 2012

Venue:
*Hotel Barceló Albatros****. Paseo de Illetas, 15. 07181 (Illetas, Calvià)*
illetasalbatros.comercial@barcelo.com

Thursday June 7th 2012

Symposium on Biomarkers & Lifestyle: Interaction Meeting BIOCLAIMS–CIBERobn

9.00-9.10h **Welcome**
Andreu Palou (BIOCLAIMS Project coordinator) &
José Antonio Fernández (CIBERobn Scientific Management Subdirector)

9.10-10.30h **Scientific sessions I: BIOCLAIMS partners**
Chairs: Lluís Arola (CTNS, Spain), Aldona Dembinska-Kiec (JUMC, Poland)

Evaluation of the BIOCLAIMS high fat diet - short term gene expression changes in white fat as predictive markers for obesity development in mice
Susanne Klaus (DIFE, Germany)

Omega 3 and metabolic syndrome
Jan Kopecky (ASCR, Czech Republic)

Nrf2 transcriptional system and biomarkers of metabolic and vascular health
Paul Thornalley (UW, United Kingdom)

10.30-11.30h Coffee Break & Poster Sessions

11.30-12.00 h **European R&D&i initiatives for the agro-food sector**
José Manuel González
CDTI. Ministry of Economy and Competitiveness.
Seventh R&D Framework Programme. KBBE Representative

12.00-13.30h **Scientific sessions II: CIBERobn partners**
Chairs: Jaap Keijer (WUR, The Netherlands), Francisca Serra (UIB, Spain)

Mediterranean diet and gene-diet interactions in determining obesity-related phenotypes
Dolores Corella (UV, Spain)

Serious Games and physical activity
Azucena García-Palacios (UJI, Spain)

Physical activity in the presence of obesity in adolescence
Julio Álvarez Pitti (UV, Spain)

13.30-15.00h Working lunch

CIBERobn Program 7 Internal Meeting (*only CIBERobn members*):

7th June, 15.00-16.30h

V BIOCLAIMS Meeting (*only BIOCLAIMS members*):

7th June, 16.30-20.30h

8th June, 9.00-18.30h

SPEAKER'S ABSTRACTS

O-01

Evaluation of the BIOCLAIMS high fat diet – short term gene expression changes in white fat as predictive markers for obesity development in mice

A. Voigt[1], E.M. van Schothorst[2], J. Keijer[2], S. Klaus[1]

[1]German Institute of Human Nutrition in Potsdam (DIfE), Nuthetal, Germany; [2]Wageningen University, Wageningen, Netherlands

Using standardized, semi-purified diets is crucial for reproducibility of experimental nutritional studies. For the purpose of comparability and integration of research two European consortia, Mitofood and BIOCLAIMS proposed an AIN-93 based standard reference diet, the BIOCLAIMS low fat diet (LFD) as well as a high fat diet (HFD). In order to evaluate these diets we performed short term (5 days) and long term (12 weeks) feeding experiments using male C57BL/6 mice. The HFD is identical to the LFD but the fat content is increased to 40% energy in exchange for carbohydrates. Both diets were well accepted and BIOCLAIMS HFD increased body fat and affected glucose homeostasis. Short term feeding trials (5 days) were performed in order to identify metabolic and molecular parameters which can serve as acute predictors for metabolic disorders due to high fat diet induced obesity. Gene expression was analyzed in gonadal white adipose tissue using whole genome microarrays. BIOCLAIMS HFD strongly influenced gene expression in white adipose tissue after short and long term intervention. A total number of 973 and 4678 transcripts showed significantly different expression between both diets after 5 days feeding and 12 weeks feeding, respectively. A total number of 764 transcripts encoding 549 genes showed significant differences between LFD and HFD after both 12 weeks and 5 days feeding. Of these overlapping genes, 79% were down-regulated and 21% were up-regulated by HFD. Pathway analysis revealed a prominent role for genes involved in lipid metabolism, carbohydrate metabolism and oxidative phosphorylation. This was confirmed by quantitative real-time RT-PCR. The high predictive value of gene expression changes in the short term study compared to long term high fat feeding suggests that a set of well defined, early biomarkers can be established which will allow a more rapid and efficient screening of potential dietary anti-obesity compounds.

Acknowledgements: The research leading to these results has received funding from the European Union's Seventh Framework Program FP7 2007-2013 under grant agreement nº 244995 (BIOCLAIMS Project)

O-02

Omega 3 and metabolic syndrome

J. Kopecky

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Obesity and associated diseases, namely type 2 diabetes, dyslipidaemia and hypertension, i.e. components of the metabolic syndrome, represent a major threat for the health care systems in affluent societies. Complex etiology of metabolic syndrome implies the need of treatments, which are based on multiple mechanisms of action. Development of the syndrome could be delayed by lifestyle modifications, while both dietary and pharmacological interventions are required for the therapy. Naturally occurring *n*-3 long-chain PUFA, namely eicosapentaenoic and docosahexaenoic acids (Omega-3), exert pronounced anti-inflammatory effects, act as hypolipidaemics, reduce cardiac events and may decrease the progression of atherosclerosis. However, Omega-3 fail to improve glycaemic control in diabetic patients. Experiments in mice fed high-fat diet revealed that Omega-3 could prevent development of obesity and hepatic steatosis, while modulating liver, adipose tissue, intestine and muscle metabolism. These effects of Omega-3 reflect changes in fatty acid composition of phospholipids, formation of Omega-3-derived lipid mediators, gene expression, as well as increases in the activity of adiponectin-AMPK axis and decrease in the activity of endocannabinoid system. Importantly, Omega-3 could augment beneficial effects of other treatments. Thus, (i) a combination treatment using Omega-3 and a mild calorie restriction efficiently reduced body fat accumulation, while inducing a metabolic switch toward lipid catabolism in adipose tissue; and (ii) a combination with anti-diabetic drugs thiazolidinediones exerted additive effects in the amelioration of dyslipidaemia and insulin resistance, while preserving muscle insulin sensitivity and metabolic flexibility, and reverting insulin resistance. Both combination treatments strongly suppressed low-grade inflammation of adipose tissue. Combination treatment using Omega-3 and a low dose of rosiglitazone reduced obesity. These results are relevant for the prevention and therapy of obesity and its comorbidities.

O-03

Nrf2 transcriptional system and biomarkers of metabolic and vascular health

M. Xue, N. Rabbani, P.J. Thornalley

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Stress responsive signalling coordinated by Nrf2 provides an adaptive response for protection against toxic insults, oxidative stress and metabolic dysfunction. Nrf2 regulates the expression of a battery of protective genes countering environment toxic insults, oxidative stress, lipid peroxidation, macromolecular damage, metabolic dysfunction and inflammation. It senses the challenge to homeostasis in the cell cytoplasm and activates a protective transcriptional response. Under basal conditions, Nrf2 transactivational activity is repressed by binding to Keap1 which holds Nrf2 in the cytosol and facilitates its degradation. Keap1 is a substrate adaptor protein for Cullin-3-dependent E2 ubiquitin ligase complex, directing Nrf2 for degradation by the 26S proteasome. Under oxidative or electrophilic stress, the Nrf2/Keap1 complex is disrupted, liberating Nrf2. Endogenous activators are lipid peroxidation and arachidonic acid oxidation products, 4-hydroxynonenal and J_3-isoprostanes, and exogenous activators are typically bioactive compounds from fruits and vegetables such as glucosinolates-derived isothiocyanates, polyphenols and flavonols, carotenoids, allyl sulphide and citrus and non-citrus triterpenoids. Nrf2 activators provide a multi-tiered protective response by countering oxidative stress, dicarbonyl stress, metabolic stress and dyslipidaemia, and activating proteasomal proteolysis and autophagic removal of damaged proteins. In the BIOCLAIMS programme we are developing and validating biomarkers of these protective responses. Experimental and clinical studies suggest that optimal exposure to dietary bioactives providing potent and sustained Nrf2-mediated transactivational activity may decrease risk and reverse early-stage development of impaired glucose tolerance, vascular dysfunction and dyslipidaemia. Novel functional foods providing effective exposure and bioavailability of Nrf2 activators will likely be important in countering the epidemic of type 2 diabetes, cardiovascular disease and obesity in Westernised and developing countries. These healthier foods may thereby provide a route to preserve and restore good metabolic and vascular health.

O-04

European R&D initiatives for the agro-food sector

J.M. González

CDTI. Ministry of Economy and Competitiveness. EU's R&D Framework Programme Department. Spanish KBBE Representative

FP7 or *Seventh Framework Programme for Research and Technological Development* is the main tool through which European Union supports research and development activities covering almost all scientific disciplines. In the Specific Programme "Cooperation" collaborative research activities on specific fields such as Food, Agriculture and Biotechnology (KBBE) are supported. We are approaching the end of FP7 and its last call for proposals (WP KBBE 2013) will allocate almost 90 M€ for research on food activities and will keep a strong focus on the need to enhance the competitiveness of the food industry and on the need to improve diets and reduce the risk of diet-related diseases. Specific priorities for WP KBBE 2013 will be brain research, understanding eating behaviour as well as solutions for vitamin D deficiency. This last call should be understood as a bridge towards the new *Horizon 2020*, which will run from 2014 to 2020. Food activities will remain at the top of the research agenda within the societal challenge "Food security, sustainable agriculture, marine and maritime research and the Bioeconomy" which proposed budget is double than its predecessor in FP7. But Europe is also looking for coordination of research activities to overcome the fragmentation and duplication of research efforts in scientific areas. As a first step, the European Commission developed the *ERA-NET scheme* in order to coordinate and support joint calls for trans-national proposals. ERANET SAFEFOODERA looked for coordination of food safety research and a follow-up of this ERANET is foreseen in the field of sustainable food production and consumption. There are further R&D initiatives such as *Joint Programming* which is an intergovernmental process aiming at pooling national research efforts in order to tackle common European challenges more effectively in a few key areas. It is worth mentioning Joint Programming of research in the field of nutrition, food and health (JPI "A Healthy Diet for a Healthy Life") that will provide for coordination at European level of research on the impact of diet and lifestyles on health, and on prevention of diet-related diseases. Lastly, *European Innovation Partnership (EIP)* as a new approach to bring innovation to the market, is aiming at speeding up the development and deployment of the technologies needed to meet the various challenges Europe is facing. Within the EIP on Agricultural Productivity and Sustainability, "Food quality, food safety and healthy lifestyles" has been identified as a specific area where innovative actions should be developed.

O-05

Mediterranean diet and gene-diet interactions in determining obesity-related phenotypes

D. Corella

Genetic and Molecular Epidemiology Unit. Department of Preventive Medicine and Public Health. School of Medicine and CIBER Fisiopatología de la Obesidad y Nutrición, University of Valencia, Valencia, Spain.

Introduction: Several studies have analyzed the interaction between some macronutrients, micronutrients or individual foods and genetic variation in candidate genes in determining obesity-related phenotypes. However, the influence of a whole dietary pattern such as the Mediterranean diet in the modulation of these associations remains to be investigated. Objective: To investigate the interaction between the Mediterranean diet, both as a whole dietary pattern and analyzing the main components of this healthy diet in determining obesity related phenotypes. Methods: We have carried out several epidemiological studies in different populations. Mainly we have focused our research in the PREDIMED (PREvencion con DIeta MEDiterránea) study, which is a multi-center clinical trial, aimed at assessing the effects of the Mediterranean diet on the primary prevention of cardiovascular disease. The total of patients included was 7,447. Participants (aged 67+/-7years) were randomly assigned to three interventions: MedDiet with extra virgin olive oil, MeDiet with mixed nuts and control group (low-fat diet). Data were analyzed both cross-sectionally and longitudinally. Validated questionnaires were used to measure diet as well as clinical and lifestyle parameters. Anthropometric, biochemical and genetic analysis were undertaken by standardized procedures. Multivariate regression models were used to investigate gene-diet interactions. Both statistical and biological gene-diet interactions were analyzed. Results and conclusions: Given that the Mediterranean diet is a complex dietary pattern, we first designed and validated a 14-item questionnaire to measure adherence to Mediterranean diet (AdMedDiet). Each question was scored 0 or 1. The greater the score, the greater the AdMedDiet. We also selected relevant candidate genes (FTO, TCF7L2, *APOA1, APOA2, ABCA1, LIPC, COX-2, PRKAG3, PRKAA2,* etc) and found interesting gene-diet interactions. In general greater AdMedDie is able to modulate the adverse effects of certain genetic variants on obesity-related phenotypes, thus protecting the genome at risk.

Acknowledgements: CNIC06, CIBEROBN, AP-042/11 and BEST11-263.

O-06

Serious Games and physical activity

A. García-Palacios[1,2], A. Cebolla[1,2], J. Alvarez-Pitti[2,5], J. Guixeres[2,5], C. Botella[1,2], R.M. Baños[2,3]

[1]Universidad Jaume I Castelló; [2]Ciber Fisiopatología de la Obesidad y la Nutrición; [3]Universidad de Valencia; [4]Unidad de Riesgo Cardiovascular. Consorcio-Hospital General de Valencia; [5]Universidad Politécnica de Valencia. I3BH Instituto interuniversitario de Bioingeniería y tecnología centrada en lo humano.

Physical inactivity has been identified as an important public health concern for children and adolescent. An increasing sedentary way of life is directly related to obesity; hence, prevention and management of childhood obesity are commonly based of lifestyle interventions wherein nutrition, physical activity (PA) and behavior modification are the main targets. Traditionally, obesity treatments have had two main goals: decreasing caloric intake through diet modification and increasing energy expenditure through promoting more active lifestyles. Promoting active lifestyles has been critical, since weight loss interventions incorporating PA are more effective at promoting long-term weight loss than diet modification alone. In fact, PA levels are the best predictors of long-term maintenance of weight loss. Although much research has focused on promoting PA in treating obese children, data still show low adherence to clinician prescriptions. In recent years, "serious games" have been developed, whose objective is the use of the player's body in the game. "Exergaming" refers to the use of video games to improve one's health and fitness. The objective of this presentation is to describe the researches made with the use of this technology in obese children, an analysis about its suitability as a clinical intervention, furthermore it will be presented some results obtained by our group in this research line. The use of active games (exergames) can be useful in promoting PA, but it is necessary to investigate the benefits these platforms can offer to obese children.

Acknowledgements: CIBERobn is an initiative of ISCIII. This research has been realized, in part, supported by Ministerio de Ciencia e Innovación (Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica 2011-2013), in the project (PSI2011-25767).

O-07

Physical activity in the presence of obesity in adolescence

J. Alvarez-Pitti[1,2]

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The prevalence of overweight/obesity (OW/OB) among children and adolescents, 40% has increased significantly in recent decades, becoming a serious public health concern in all industrialized countries. This increase has immediate- and long-term health implications. Although prevention is recognized as the primary and most efficient way to avoid obesity, many children and adolescents who are currently obese require treatment. Essentially, the major objectives of a weight-reduction programme are to change food and behavioural habits and to enhance physical activity (PA). The addition of PA to dietary changes has proven to be beneficial improving body composition, blood pressure levels, lipid profile, insulin sensitivity, self esteem, neurocognitive function and cardio-respiratory fitness (CRF). There are emerging data to suggest that CRF attenuates some of the factors contributing to metabolic syndrome in adolescence, oftentimes independent of adiposity. In order to improve all this conditions, the WHO has recently published the recommended levels of physical activity for health. These recommendations includes for children and young people aged 5-17 years old, physical activity that should accumulate at least 60 minutes of moderate-to vigorous-intensity physical activity daily. Benefit of PA in obesity treatment seems to be clear, but the results of the evidence show us that the optimal exercise modality that should be recommended for its treatment is unclear. The impossibility to perform the kind of PA usually recommended (type, duration, frequency and intensity), the lack of clear statement about its specific goal and the fact that prescribed physical activity is not enjoyable, are some of the reasons described in the literature to explain the low compliance and efficacy of paediatric obesity treatment programmes. It is necessary to explore new ways to prescribe PA as part of the treatment of obesity in children and adolescents taking into account orthopaedic, fitness and behavioural particularities of these populations.

POSTER ABSTRACTS

P-01

Videogame therapy in Eating Disorders: An innovative approach for regulating emotions

Z. Agüera[1,2], S. Jimenez Murcia[1,2,3], J. Santamaria[1,2,4], L. Forcano[1,2], I. Sanchez[1], N. Riesco[1], Playmancer Consortium[4], F. Fernandez-Aranda[1,2,3,4]

[1]Department of Psychiatry, University Hospital of Bellvitge-IDIBELL, Barcelona, Spain; [2]CIBER Fisiopatología Obesidad y Nutrición (CIBERObn), Instituto Salud Carlos III, Spain; [3]Clinical Sciences Department, Medicine School, University of Barcelona, Spain; [4] www.Playmancer.eu

Objectives: Based on the current difficulty to treat specific areas (e.g. some personality traits, attitudinal and emotional aspects, and uncontrolled behaviors), in Eating Disorders (ED), the purpose of this pilot study was to test the effectiveness of using a specific designed videogame (Playmancer) for treating ED, when compared with controls. Within this serious game, several components are interacting and influencing subject's performance, such as physiological reactivity and emotional expression (speech and face). Method: 15 BN/ BED patients and 15 healthy eating controls were compared in this study. All the subjects were females and the clinical cases were diagnosed according to DSM-IV criteria. Following a baseline design (A-B-A), physiological reactivity (heart rate –HR-, breathing, oxygen saturation, galvanic skin reaction, pulse rate, temperature) and emotional expression in front of this serious game were tested. Assessment measures included the BIS-11, STAI and GHQ-28, as well as a number of other clinical and psychopathological indices. Results: In both groups, significant differences among phases (A-B-A) were observed in all physiological measures ($p<.005$). Along the videogame, ED showed higher respiration rates ($p<.028$) and lower HR variation ($p<.018$). Differences among the groups were found along the videogame on emotional expression (e.g. anger). Conclusions: After using specific task with this videogame, good physiological reactivity has been observed, as well in ED cases as in the comparison group. ED overemphasized their physiological reactivity and emotional expression when compared with controls. Playmancer, seems to be a good tool for triggering specific emotions in ED.

Acknowledgements: Support by the PlayMancer EU project (FP7-ICT-215839-2007), which was funded by the FP7 of the European Commission. The project received also partial support from ISCIII (CIBER06/03, FIS PI081573). CIBERobn is an ISCIII initiative.

P-02

Home-Based Exercise Programme: A novel approach of childhood obesity treatment

J. Alvarez-Pitti[1,3], J.F. Lisón[2], J.M. Real-Montes[2], I. Torro[1,3], M.D. Arguisuelas[2], L. Cantero[3], F. Aguilar[1,3], E Lurbe[1,3]

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Introduction: the prevalence of overweight/obesity (OW/OB) among children and adolescents, has increased in recent decades. Although there is agreement within the scientific community that exercise is an empirically validated method of treating obesity, the optimal exercise modality that should be recommended for its treatment is unclear. A novel approach that has not been entirely explored is the home-based exercise programs. Objective: The aim of this study was to compare the effect of a hospital group-vs. home-based combined exercise-diet program for the treatment of childhood obesity. Methods: One hundred and ten OW/OB Spanish children and adolescents (6-16y) falling in two intervention groups (hospital group-based (n=45) and home-based (n=41)) and a sex-age-matched control group (n=24), were randomly assigned to participate in a 6 month combined exercise (aerobic + resistance training) and Mediterranean diet program. Anthropometric values (including body weight, height, body mass index (BMI), BMI-Z score, and waist circumference) were measured pre- and post-intervention for all the participants. Percentage body fat was also determined using a body fat analyzer (TANITA TBF-410 M). Results: Our study showed a significant reduction in percentage body fat and BMI Z-score among both intervention-group participants (4%, 0.16, hospital group-based; 4.4%, 0.23, home-based; $p<0.0001$). There was also a significant reduction in waist circumference in the home-based group (4.4 cm; $p = 0.019$). Attendance rates at intervention sessions were equivalent for both intervention groups ($p = 0.805$). Conclusions: The study findings indicate that a simple home-based combined exercise and Mediterranean diet program may be effective among overweight and obese children and adolescents, because it improves body composition, is feasible and can be adopted on a large scale without substantial expenses.

Trial registration: Clinicaltrials.gov NCT01503281.

P-03

Search of early biomarkers of metabolic robustness in the model of mice supplemented with physiological doses of leptin during lactation

M. Asnani, A.M. Rodríguez, A. Palou

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Leptin supplementation with physiological doses during lactation protects from obesity development and associated disorders in male adult rats. It is of interest to check the model in other mammals, to study the sex-dependent responses and to look for early biomarkers suitable of predicting the metabolic outcome in adults. We aimed to study the effects of leptin supplementation during lactation in male and female mice, studying the possible protection from obesity development (and associated disorders) under a high-fat diet. In a pre-study, leptin quantity in murine milk was estimated. In the main experiment, pups were divided in control and leptin-treated offspring. Leptin treatment was supplemente with a physiological dose of leptin (x3 the mean quantity estimated) from day 2 to day 20 of lactation. At 11 weeks age, both control and leptin-treated animals were divided in: low(3%)-fat and high(45%)-fat diet, until 6-month age, when the animals were sacrificed and key tissues collected. Throughout the experiment, body weight and composition (by MR) were monitored, and plasma samples were collected at fed and fasting conditions in key time-points. Non-leptin treated males reached a significant higher body weight and fat percentage under high-fat diet, while no differences were shown between high-fat and low-fat males treated with leptin. The percentage of body fat was higher in low-fat leptin-treated than in low-fat control males, but lower than in control high-fat males. Females increased their body fat under high-fat diet, without differences in the leptin and non-leptin-treated groups. The preliminary results point out a possible protective effect of leptin treatment under a high-fat diet in males and key differences between sexes. The model stands out as good for the search of molecular differences responsible of the leptin and sex-dependent responses and for early biomarkers in the blood samples collected during the experiment.

P-04

Description of an Exergame specially designed for treatment of childhood obesity: Drappen's world

R.M. Baños [1,3], A. Cebolla[2,3], J. Guixeres[1,4], P. Escobar[1,2], L. Vara[1], E. Oliver[1,3]

[1]Universidad de Valencia; [2]Universidad Jaume I Castellón; [3]Ciber Fisiopatología de la Obesidad y la Nutrición; [4]Universidad Politécnica de Valencia. I3BH Instituto interuniversitario de Bioingeniería y tecnología centrada en lo humano.

The pattern of physical exercise is an essential part of clinical intervention protocol for childhood obesity. However, the levels of adherence to this component are very low. This fact can be explained in part by factors such as low motivation, low satisfaction or low physical tone that obese patients present with respect to physical exercise. Information technologies and communication platforms like Exergaming can help develop intervention programs aimed to help overcome these barriers. Exergaming refers to games whose objective is the involvement of the body in the game to improve health and fitness. Exergaming has the advantage of previous experiences with children and are designed to allure and adapt to the level of effort that the subject requires, in order to become a powerful reinforcer. The aim of this poster is to describe an Exergame called "Drappen's World" specifically designed for the promotion of physical activity and exercise in obese children. The argument is that the gamer has to take care of a Drangon's egg. The gamer has to play various games which imply different physical activity movements. The game uses a "Kinect Microsoft's" technology to move the character. This game also has an Android application with GPS connection, thus the gamer has to walk 2 Km in order to get a key and continue the game after a period of time. This game will be inserted into a childhood obesity treatment intervention (ETIOBE) as a complement to improve the adherence to physical activity. It is expected to be well received by users and it will improve the efficacy of the traditional interventions to promote Physical activity in Obese Children. Exergaming platforms have great potential as a tool for promotion and enhancement of physical activity.

Acknowledgements: CIBERobn is an initiative of ISCIII. This research has been realized, in part, supported by Ministerio de Ciencia e Innovación (Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica 2011-2013), in the project (PSI2011-25767).

P-05

Indirect calorimetry assesment of metabolic flexibility in A/J and C57BL/6J mice

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Our aim was to learn whether metabolic flexibility could serve as a biomarker of susceptibility to obesity. Thus, indirect calorimetry was used to compare metabolic flexibility in obesity-prone C57BL/6J and the obesity-resistant A/J mice one day after weaning to chow diet (at 30 days age). Mice were maintained at 22^{o}C, while the measurement was performed at 34^{o}C. Two protocols were used for indirect calorimetry. First, the measurement was performed in mice fasted during the light phase of the day, and then re-fed chow during the night. Using this approach, ambiquos results were obtained, due to a higher intake of chow diet during the night in the A/J mice. Second, to exclude the influence of different caloric intake we further assessed the metabolic response to the bolus of glucose (7.5 μg of glucose per g of body weight) applied intragastrically after 4 hours of fasting. The rate of the increase in RQ values induced by the glucose administration was quantified using a Hillslope parameter of RQ curve (by interspacing a dynamic fit curve into individual RQ data from time interval 1 hour before intragastric gavage to 1.5 hour after gavage from each mice). This parameter was higher in A/J as compared with C57BL/6J mice, independent of the gender. In conclusion, C57BL6/J mice exerted a relatively low ability to switch between metabolic substrates, whereas A/J mice exhibited a relatively high metabolic flexibility to glucose. Metabolic flexibility shortly after weaning might represent a biomarker of genetically-determined obesity.

P-06

Impact of calcium and conjugated linoleic acid on the gastrointestinal tract in mice

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Introduction.- Both calcium and conjugated linoleic acid (CLA) are potential beneficial compounds in body weight management. We have shown that induced obesity with a high-fat diet can be counteracted by calcium supplementation and that moderate doses of CLA contribute to lower body weight gain and fat accretion in mice. Objective.- The aim of this study was to assess the potential impact of both compounds in the gastrointestinal tract in mice. Methods.- Mice (C57BL/6J) were divided into five groups according to diet and treatment (up to 56 days): control (C), high-fat diet (HF), HF with calcium (Ca), HF plus CLA (CLA) and HF with both (Ca+CLA). At the end of treatment, expression of selected gastric peptides was determined in the stomach by qPCR. Studies on the characteristics of caecum microbiota have been initiated by qPCR. Results.- Our results indicate that calcium supplementation in the high-fat diet seems to regulate gene expression similar to control levels, whereas CLA treatment is associated with a general increase in the expression of the gastric peptides studied (leptin, ghrelin, Grp39, glucagon and its receptor and resistin). Furthermore, both compounds are associated with significant effects on the intestinal microbiota. Our data indicate a significant increase in bacterial DNA in the HF mice that tends to decrease by CLA and recover control values by calcium supplementation. This is accompanied by differences in some of the most predominant bacterial genes amongst groups. Further research is underway to outline the main microflora adaptations found associated to these compounds and their potential as fat-lowering agents.

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P-07

Gene expression profile of peripheral blood mononuclear cells (PBMC) is affected by diet macronutrient composition

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The intake of diets with an unbalanced proportion of macronutrients, mainly fats and proteins, is associated with different pathologies (e.g. diabetes, obesity, CVD, cancer, etc.). The study of the metabolic effects of unbalanced diets plays a central role in the understanding of these pathologies. Peripheral blood mononuclear cells (PBMC) are readily accessible biological material which use for nutrition studies is increasing as they can reflect gene expression of other internal tissues. The aim of this study was to assess the effect of the intake of isocaloric high-fat/high-protein diets on PBMC gene expression of 6-month-old male Wistar rats using microarray analysis, focusing on the analysis of the altered pathways. For that purpose, 2-month-old male Wistar rats were fed for 4 months with a control normal-fat (NF; 70% of calories from carbohydrates, mainly corn starch), a high-fat (HF; 60% of calories from fats, mainly lard) or a high-protein (HP; 45% of calories from proteins, mainly casein) diets in isocaloric amounts (pair-feeding). Our results of microarray analysis showed that 113 and 323 genes of a total of 21,530 probes changed as a result of the intake of the HF and the HP diets, respectively (Student's t test, $p<0.05$ and absolute fold change ≥ 1.2). Pathways mainly affected by HF diet were: cell cycle, gene expression, immune response and cell communication/signal transduction. HP diet affected mainly to: cell communication/signal transduction, gene expression and immune response Moreover, 10 genes were simultaneously and equally altered by both diets. In conclusion, multiple pathways involved in several pathologies and metabolic disorders are affected by diet macronutrient composition (HF/HP diets). Thus, PBMC could be used as a biological source to detect markers of the intake of diets with an unbalanced proportion of macronutrients.

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P-08

Analysis of physical activity and intake habits in overweight and obese children sample from Cardiovascular Risk Unit of a Hospital

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A healthy lifestyle involves both a balanced intake and a regular physical activity. All obesity prevention and treatment programs are focused on reducing the amount of calories in diet and increasing exercise. In order to establish adequate targets in obesity interventions is necessary to analyze the physical activity and intake habits. Regarding intake, information about consumption of fruit and vegetables is especially relevant because their intake may displace the consumption of energy-dense, nutrient-poor foods associated with childhood obesity (Epstein et al., 2001). The goal is to know the lifestyle habits of a specific sample of overweight and obese adolescents regular users of a cardiovascular risk unit of a Hospital in Valencia, in order to develop strategies ad-hoc to characteristics of this sample and promote healthy lifestyle. The sample was composed by 36 children (9-15 years old), from a Paediatric Service located in a public hospital specializing in childhood obesity treatments. All participants fulfilled two questionnaires: the KIDMED (Serra-Majem et al., 2004) to evaluate the intake of fruits, vegetables and fats, and Physical Activity Questionnaire for Adolescents (PAQ-A) (Martínez-Gómez, et al., 2009) to evaluate the physical activity daily life. Regarding intake habits, results show that children consumed at least one piece of fruit at day (91.7%), at least one portion of vegetables at day (77.8%) and 72.2% indicated that they have a healthy breakfast with milk, cereals, fruit, etc. Regarding physical activity, most children informed that they attend to physical activity classes at school, but in leisure time, they preferred sedentary activities. According to our results**,** children under obesity treatment follow a healthy diet, but they show a sedentary lifestyle. It seems that the diet interventions in hospitals are adequate but it should be important to develop more strategies that promote physical activity in daily life.

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P-09

Executive functions profile in extreme eating/weight conditions: from anorexia nervosa to obesity

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Introduction: Extreme weight conditions (EWC) groups along a *continuum* may share some biological risk factors and intermediate neurocognitive phenotypes. A core cognitive trait in EWC appears to be executive dysfunction, with a focus on decision making, response inhibition and cognitive flexibility. Differences between individuals in these areas are likely to contribute to the differences in vulnerability to EWC. Objectives: The idea behind the study was to investigate whether there is a common pattern of executive dysfunction (decision making, cognitive inhibition and cognitive flexibility) in EWC while comparing anorexia nervosa patients (AN), obese subjects (OB) and healthy eating/weight controls (HC). Methods: Thirty five AN patients, 52 OB subjects and 137 HC were compared using using a neuropsychological battery including the Wisconsin Card Sorting Test (WCST); Stroop Color and Word Test (SCWT); and Iowa Gambling Task (IGT). All participants were female, aged between 18 and 60 years and spoke Spanish as their first language. Results: There was a significant difference on IGT score (F (1.79); p<. 001), with AN and OB groups showing the poorest performance compared with HC. On the WCST, AN and OB made significantly more errors than controls (F (25.73); p<. 001), and had significantly fewer correct responses (F (2.71); p <. 001). Post hoc analysis revealed that the two clinical groups were not significantly different from each other. Finally, OB showed a significant reduced performance in inhibition response measured with the Stroop test (F(5.11); p<. 001) compared with both AN and HC. Conclusion: These findings suggest that EWC subjects (namely AN and OB) have similar dysfunctional executive profile that may play a role in the development and maintenance of such disorders.

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P-10

Early alterations in plasma ghrelin levels in rats predisposed to obesity seems linked to lower sympathetic drive to the stomach

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Serum ghrelin concentration is reduced in obesity. We aimed to assess whether this alteration is present in rats predisposed to obesity because of moderate undernutrition during gestation, and to explore whether this could be related with alterations in stomach sympathetic innervation, which is involved in gastric ghrelin secretion. Offspring of control and 20% gestational calorie-restricted dams (CR) exposed to normal-fat-diet from weaning onward were studied. Circulating ghrelin levels were measured at 25days and 4month of age. Morphometry, number of ghrelin-positive (ghrelin^{+}) cells, ghrelin mRNA and protein levels, and tyrosine hydroxylase (TH) protein levels in stomach were determined at 25d. Adult CR male animals, but not females, exhibited greater body-weight (BW) than their controls, but both males and females showed lower circulating ghrelin levels. This alteration was already present at 25d, prior to any difference in BW. At this juvenile age, no differences in gastric morphometry, number of ghrelin^{+} cells or ghrelin mRNA/protein levels were found between control and CR animals; however, CR animals showed lower TH stomach content; suggesting that circulating ghrelin concentration is early altered in rats prenatally programmed to develop obesity. This does not seem to be associated with lower ghrelin production capacity but with specific alterations in sympathetic drive to the stomach.

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P-11

Liver response to supplementation with high esterified pectins in obesity prone rats

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Introduction/objective: Prebiotics can help to combat obesity and its associated disorders. We studied the effects of supplementation with high esterified pectins (HEP) from apple in the obesity-prone rat model CR (offspring of moderate caloric-restricted dams during pregnancy) under normal and high sucrose diet. Methods: After weaning, male offspring of 20% caloric-restricted dams (pregnancy days 1-12), were fed with a standard diet only (CR) or supplemented with 10% HEP (CR-P) until 4-month age, then half of each group was also supplemented with 30% sucrose (CR-S/CR-P-S). Fed and fasting circulating glucose and insulin were measured (5-month age) and insulin resistance was assessed by HOMA score. Liver mRNA and protein levels of lipolytic/lipogenic genes were measured by RT-qPCR and by Western blot. Results: HEP-supplemented rats (CR-P/CR-P-S) showed significantly lower body weight, fasting glucose, triglycerides and insulin levels and HOMA score than their controls (CR/CR-S). The most outstanding mRNA expression results were: increased mRNA expression of ATGL, PGC1α and AMPKα2 under sucrose in HEP-supplemented rats and increased levels of ACC1 only in the sucrose group, expression of FASN was decreased by HEP supplementation. There were generally higher mRNA levels of CPT1a in the HEP supplementation group. Protein levels of total AMPKα and ACC were in concordance with mRNA expression levels. Conclusion: Results suggest higher capacity of circulating glucose and triglycerides clearance under HEP supplementation accompanied by a possible higher capacity for lipolysis and oxidation in liver. HEP can help to improve body weight and key metabolic parameters in the obesity-prone model of CR rats.

P-12

High-fat isocaloric pair-feeding affects the expression of AgRP and CART, hypothalamic peptides involved in food intake control

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Excessive intake of hipercaloric high-fat diets is one of the main causes of obesity. Our objective was to assess the effect of feeding with a high-fat isocaloric diet compared to a control diet on the expression of key hypothalamic neuropeptides implicated in the control of food intake. Male Wistar rats were pair-fed (isocaloric feeding) for 4 months with a normal-fat (NF) and a high-fat (HF) diet –10/60% calories from fat, respectively. Body weight and composition and food intake were periodically registered. Glucose, insulin, leptin and ghrelin levels were measured in serum. mRNA expression of hypothalamic neuropeptides related to food intake was measured by Q-PCR. Our results indicated that high-fat pair-feeding did not affect body weight but resulted in a higher adiposity and increased insulin resistance as reflected by higher HOMA index. Although daily caloric intake was not affected, HF-feeding produced an increase in mRNA expression of the orexigenic peptide agouti-related protein (AgRP) and a decrease in the anorexigenic peptide cocaine and amphetamine regulated transcript (CART). No change was observed in the expression of the orexigenic neuropeptide Y (NPY) and anorexigenic pro-opiomelanocortin (POMC). Serum levels of the orexigenic signal ghrelin were increased as a result of the HF-feeding while no change was observed in circulating levels of the satiating signal leptin. In conclusion, an increased fat proportion in an isocaloric diet produces a stimulation of appetite as evidenced by a higher AgRP/CART ratio and higher basal circulating ghrelin levels. This fact could contribute to an increase in body weight if animals were given free access to food.

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P-13

Preservation of Metabolic Flexibility in Skeletal Muscle by a Combined Use of *n*-3 PUFA and Rosiglitazone in C57BL/6N diet-induced obesity mice

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Impaired sensitivity to insulin, the key defect in type 2 diabetes, is associated with a low capacity to adapt fuel oxidation to fuel availability, i.e., metabolic inflexibility. The hampered metabolic adaptability triggers a further damage of insulin signaling. We have shown previously in mice fed an obesogenic high-fat diet with a combined use of *n*-3 long-chain polyunsaturated fatty acids (*n*-3 PUFA) and thiazolidinedione (TZD) antidiabetic drugs, rosiglitazone and pioglitazone, preserved metabolic health and synergistically improved muscle insulin sensitivity. We investigated here whether a relatively low dose, rosiglitazone could elicit the additive beneficial effects on metabolic flexibility when combined with *n*-3 PUFA.

Adult male C57BL/6N mice were fed an obesogenic high-fat diet (cHF; 35 g lipids/100 g diet) for 8 weeks, or randomly assigned to various dietary interventions: (i) cHF+F, cHF with *n*-3 PUFA concentrate replacing 15% of dietary lipids; (ii) cHF+ROSI, cHF with 10 mg rosiglitazone/kg diet; and (iii) cHF+F+ROSI. Only the combined use reduced body weight gain (cHF, 11.7 ± 1.3; cHF+F+ROSI, 6.8 ± 0.9 g; $p<0.05$). Indirect calorimetry demonstrated superior preservation of metabolic flexibility to carbohydrates in response to the combined use. Targeted metabolomic analysis and gene expression analyses in the muscle suggested distinct and complementary effects of the single treatments, with rosiglitazone augmenting insulin sensitivity by the modulation of branched-chain amino acid metabolism, and *n*-3 PUFA supporting complete oxidation of fatty acids in mitochondria. These beneficial metabolic effects were associated with the activation of the switch between glycolytic and oxidative muscle fibers, especially in the cHF+F+ROSI mice. Most of these changes were independent of the anti-obesity effect of the combined use.

Our results further support the idea that the combined use of *n*-3 PUFA and classical TZD drugs, or novel drugs in this class, could improve the efficacy of the treatment of obese and diabetic patients.

P-14

Impact of maternal smoking on methylation signature in low birth weight newborns

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The intrauterine development is a critical period of developmental programming in the human life. A large body of epidemiological studies support a clear link between an adverse intrauterine environment and an increased risk to develop cardiometabolical diseases in later adult life, like obesity, type 2 diabetes, and hypertension. Maternal smoking during pregnancy has been identified as the most important determinant of birth weight in developed countries. Smoking is clearly associated with slow pre- and postnatal growth, and with a higher risk of having low birth weight children. It also increases the risk of asthma during the first seven years of life, the risk of overweight and the risk of cognitive and neurodevelopmental delay. The mechanism by which the intrauterine environment induce disease propensity is beginning to be understood, and epigenetic modifications have emerged as potential molecular mediators. Our objective is to determine whether the effects of the maternal smoking during pregnancy leads to changes in the methylation status in low birth weight newborns (LBW) and to compare these epigenetics changes with LBW newborns without prenatal tobacco exposure. DNA methylation was analyzed in umbilical cord blood cells from 10 LBW newborns from smoking mothers and compared with 10 LBW newborns non exposed. The assessment of methylation status was determined using Illumina Human BeadsChips 450K, and comparative analysis of LBW prenatally exposed *vs* LBW non exposed revealed that global DNA methylation levels were similar between two groups (Average beta 0.64± 0.14, non exposed vs 0,62±0,16 exposed). Howewer, we observed that 114 CpG sites are significant differentially methylated (cutt-off of delta beta ≥0.2; Diff score≥ 300) showing hypermethylation in loci involved in cell cycle control, immune response, diabetes and asthma. These results suggest that the epigenetic processes are differentially deregulated in LBW newborns exposed prenatally to tobacco compared to those non exposed.

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P-15

Identification of early markers of improved metabolic functions of brown and white adipose tissues in the offspring of calorie-restricted dams during lactation

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We have previously shown that moderate maternal caloric restriction in lactating dams improves metabolic health of their offspring by conferring certain protection against the development of obesity and its related metabolic pathologies in adulthood, particularly dyslipidemia, insulin resistance, and hyperleptinemia, associated with HF diet feeding. Here we aimed to search for early markers of this programming effects occurring in brown and white adipose tissue at the gene expression level that may contribute to explain the protective effects of this postnatal condition. The offspring (males and females) of control and 20% caloric restricted dams throughout lactation (CR) were studied. At weaning, part of the pups were sacrificed under feeding conditions and the retroperitoneal white adipose tissue (WAT) and the interscapular brown adipose tissue (BAT) were collected to perform gene expression analysis (by RT-qPCR). The remaining animals were kept alive on a normal fat (NF) diet and were followed until the age of 4 months. CR male and female animals showed in adulthood lower body weight than their controls, and this was associated in females with lower fat accretion. At weaning, CR pups displayed in the WAT higher mRNA levels of genes related with lipolysis (ATGL, PPARá) and of insulin signaling (InsR, IRS1) and lower mRNA levels of leptin and lipogenesis–related genes (FAS, GLUT4 and GPAT, the two latter only in females) in comparison to controls. CR animals also showed higher mRNA levels of CPT1 and UCP1 in BAT. These results suggest that the improvement of metabolic health related with moderate maternal caloric restriction during lactation may be associated, at least in part, with early programming effects in the metabolic capacity of the adipose tissues, affecting its adipogenic, oxidative and thermogenic capacity.

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P-16

The impact of CLA in lipogenic enzyme expression is attenuated in presence of dietary co-supplementation with calcium in mice

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Introduction: Epidemiological studies suggest that increased calcium intake helps to reduce body weight and adiposity. On the other hand, the conjugated linoleic acid (CLA) shows similar effects, particularly in obese animals. Objective: The aim of this study was to assess the potential synergistic effect of both calcium and CLA on body weight and fat in mice. Materials and methods: Mouse (C57BL/6J) were divided into five groups according to diet and treatment (up to 56 days): control (C), high-fat diet (HF), HF with calcium (Ca), HF plus CLA (CLA), and HF with both (Ca+CLA). At the end of experiment, expression of fatty acid synthase (FAS) and stearoil CoA desaturase (SCD1) were determined by q PCR in adipose tissue, liver and muscle. Results and conclusions: Both, Ca and CLA supplementation contributed to lower body fat gain under HF diet. The minimum body fat content was seen by Ca supplementation and no additional effect was associated with the combined treatment (Ca+ CLA). In adipose tissue, FAS and SCD1 were induced by CLA (2-3 fold), whereas were reduced by Ca (30% and 70%, respectively in comparison with controls); combined treatment of CLA+Ca was associated with a moderate induction of expression in both enzymes (1.4 fold in FAS and 2.4 fold in SCD1). In muscle and liver, the trend was different: CLA treatment tended to reach control values; Ca supplementation was accompanied by lower levels in comparison with controls (60-70% in FAS, 20-40% in SCD1) and effects of the combined treatment were closer to Ca effects. In conclusion, both dietary calcium and CLA may contribute to counteract obesity in mice. At the doses assayed, no synergistic effects were seen and tissue-specific responses could be underlined. Particularly, adipose tissue is highly sensitive to CLA whereas liver and muscle are more sensitive to Ca action.

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P-17

The n-3 PUFA supplementation obesity, and posprandial GIP concentration (The Bioclaims Study)

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Intorduction: The influence of n-3 PUFA on development of T2D remains unresolved. In patients with glucose abnormalities the *glucose-dependent insulinotropic peptide* (GIP) secretion is normal or even higher. The aim of the study: Was to evaluate the effects of n-3 PUFAs supplementation on glucose and lipid metabolism and BMI in obese and non-obese volunteers with/without caloric restriction after oral glucose or fat challenges. Methods: 50 patients aged 25-65 years, has been randomly assigned to 3 subgroups of diets: control (BMI≥25 ≤29kg/m2) and obese (BMI≥30 ≤40kg/m2) with isocaloric and low-calorie diet. Patients from each subgroup were divided on placebo or on 3x600 mg capsules (1800mg/day) n-3PUFA, with DHA:EPA ratio 5:1 (EPAX 1050TG) supplementation. Before and after 3 months of diet and PUFA / placebo supplementation participants undergone the oral glucose tolerance test (OGTT) and oral lipid tolerance test (OLTT). The blood insulin, FFA, TG and GIP concentrations were measured. Results: After 3 months of intervention patients with EPAX supplementation on low-calorie diet achieved significant reduction of BMI as well as TG levels The increased postprandial GIP AUC (p=0,05) during OLTT but not OGTT was observed. Conclusion: Considered GIP action, we can assume that n-3 PUFA supplementation may have impact on glucose homeostasis especially after postprandial lipid load. The clinical relevance of this relation and its possible mechanisms require further investigation

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Effect of leptin on the expression of transcription factors and coactivators involved in oxidative metabolism in C2C12 muscle cells: time course study comparing different supra- and physiological concentrations

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Introduction: Leptin is involved in energy metabolism regulation, acting on different peripheral tissues, such as skeletal muscle. Peroxisome proliferator-activated receptor (PPAR) gamma coactivator 1 (PGC1) family is highly expressed in tissues with high energy metabolism. They coactivate transcription factors, as PPARs, regulating genes engaged in processes such as mitochondrial biogenesis and oxidative metabolism, among others. Objective: To progress in the present understanding of leptin effect on muscular cells, by comparing the effect of physiological and supraphysiological concentrations of leptin on the expression of PGC1α and PPARs in C2C12 myocytes. Methods: Cells were treated with 5, 10 and 50ng/ml of leptin for 0, 30', 3h, 6h, 12h and 24h. Time-course changes in gene expression in non-treated cells were also monitored. mRNA levels of PGC1α, PPARα, PPARβ/δ, and PPARγ were analyzed by RT-qPCR. Results: With respect to time controls, 50ng/ml leptin significantly induced the expression of PGC1α at the short-term (30'/3h), with a tendency for PPARα (also with 5ng/ml) and PPARγ. Moreover, the concentration of 5ng/ml significantly induced the expression of PGC1α at 12h and 24h. On the contrary, PPARγ expression was reduced with 50ng/ml leptin at the long-term (24h). Conclusion: Leptin can induce the expression of transcription factors and coactivators related with the induction of oxidative capacity in muscle cells, being outstanding the induction of the master coactivator PGC1α, even at low (physiological) leptin concentrations.

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SIRT1 expression as a potential early biomarker of obesity susceptibility in offspring of calorie-restricted rats during gestation

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We have previously shown that 20% caloric restriction during gestation programs offspring for higher food intake, which in adulthood results in higher body weight in males but not in females. Here, we aimed to assess whether the gender-dependent outcomes of this condition during gestation on adult body weight may be related with metabolic programming of sirtuin (SIRT) expression in different tissues. For this purpose, 25-day-old offspring of control and 20% caloric-restricted rats (from days 1-12 of pregnancy) (CR) were studied. Body weight and weight of retroperitoneal WAT (rWAT) and liver were recorded and mRNA expression of SIRT1 and other selected genes in rWAT, liver, skeletal muscle and hypothalamus were analyzed. No differences were found in body weight or weight of rWAT and liver between control and CR animals. Interestingly, a similar pattern of SIRT1 mRNA expression was found in rWAT, liver and muscle in CR animals, but in a sex-dependent manner: CR males showed lower SIRT1 mRNA levels than controls, while no differences were found in females. A sex-different pattern was also observed in hypothalamus. CR males, but not females, also showed lower mRNA levels of ATGL and UCP2 in rWAT and of SREBP1c and SCD1 in liver. Both male and female CR animals showed lower AMPK and ATGL mRNA levels in liver. In summary, 20% maternal caloric restriction during gestation programs a gender-dependent gene expression profile of SIRT1 in different tissues, which may be related with obesity predisposition in adulthood; therefore SIRT1 expression emerges as a potential early biomarker of obesity susceptibility.

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P-20

Differential expression profile of microRNAs in gastrocnemius muscle in response to CLA and calcium-supplemented high-fat diet

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Introduction.- MicroRNAs (miRNAs) are short non-coding RNAs that regulate target gene expression mainly at post-transcriptional level in several biological processes. The analysis of miRNAs expression offers a promising tool for therapeutic research. Objective.- The aim of this study was to investigate whether miRNAs expression profile can reflect the potential impact of a dietary nutrient supplementation in the prevention of obesity. Methods.- C57BL/6J wild type male mice were divided into four groups according to diet and treatment (up to 56 days): control diet (C), high-fat diet (HF), HF with calcium (Ca) and HF plus conjugated linoleic acid (CLA). At the end of the experiment, RNA from gastrocnemius muscle was isolated for miRNAs profiling. Results.- Muscle miRNAs expression reflected the differential effects of calcium and CLA supplementation associated with the fat lowering effects of both compounds. Calcium supplementation was associated with a decrease of body weight and body fat comparable to the control group and resulted in changes in the expression of 127 miRNAs, whereas miRNAs expression changes in CLA group was smaller (15 miRNAs) according to the slight reduction in fat accumulation observed. Conclusion.- These preliminary results show the impact of dietary compounds in the expression of muscle miRNAs and its potential functional role in slimming strategies. Furthermore, the data give support to the contribution of miRNAs as novel biomarkers for assessing the potential impact of a nutrient supplementation in the prevention of obesity.

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Differential responses to metabolic stressors in mice haploinsufficient for the retinoblastoma protein gene

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Introduction: Inactivation of the retinoblastoma protein (pRb) favors brown adipogenesis and protects against diabesity in animals by increasing oxidative metabolism in fat tissues. Objective: Here, we compared Rb haploinsufficient ($Rb^{+/-}$) and wild-type (WT, $Rb^{+/+}$) mice in front of ageing and conditions of acute, nutritional/metabolic stress. Methods: $Rb^{+/-}$ and WT mice fed a standard diet were studied from weaning until the age of 6-7 months. The following was performed: follow-up of body weight and energy intake; body composition analysis, HOMA-IR index, glucose tolerance test (GTT) and insulin sensitivity test (ITT) at 2-3 months and 6 months of age; indirect calorimetry at 7 months of age; oral fat tolerance test and leptin sensitivity test at 2 months of age. Results: At 2-3 months of age, no differences between Rb genotypes regarding body weight and body composition were observed. However, young $Rb^{+/-}$ mice showed increased sensitivity to the anorexic effect of exogenous leptin and increased fat tolerance compared with WT mice. At 6-7 months of age, $Rb^{+/-}$ mice displayed reduced body fat and reduced respiratory quotient, indicating a greater use of fatty acids as a fuel. Additionally, both at the juvenile and the older age studied, $Rb^{+/-}$ mice displayed signs of increased sensitivity to insulin (in ITT) and increased glucose tolerance (in GTT). With ageing, insulin sensitivity measured as HOMA-IR index significantly deteriorated in the WT mice, but not in the $Rb^{+/-}$ mice. Conclusion: The results reinforce the involvement of pRb in metabolic control, and suggest that Rb haploinsufficiency may confer metabolic advantages in front of metabolic stressors.

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Pocket protein expression in association with obesity in human and rat adipose tissue

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Introduction**:** Retinoblastoma protein (pRb), the well-known tumor suppressor, has been described as an essential player in murine white adipocyte differentiation and a negative modulator of brown adipocyte differentiation. To our knowledge, no studies have explored pRb expression in connection with established obesity in humans or rodents. Objective: To investigate gene expression of the retinoblastoma protein family members (pRb, p130 and p107) in human and rat adipose tissue according to obesity and insulin sensitivity. Methods: *Human studies*: Gene expression of pRb, p130 and p107 was analyzed by real time PCR in two independent human cohorts, in 154 adipose tissue samples (77 visceral and 77 subcutaneous) from participants with different degrees of obesity (first cohort), and in 64 adipose tissue samples (32 visceral and 32 subcutaneous) from morbidly obese participants with different degrees of insulin action (measured with euglycemic clamp) (second cohort). *Rodent studies*: Gene expression of pRb, p130 and p107 was analyzed by real time PCR in visceral (retroperitoneal, rWAT) white adipose tissue of male Wistar rats fed for 8 weeks, from month 2 to month 4 of age, a normal fat diet (10% of energy as fat, control rats), a high fat diet (60% of energy as fat) or a cafeteria diet, and in a group of male Wistar rats fed cafeteria diet as above and then a normal fat diet up to the age of 6 months. Pearson's correlations between pocket protein expression and parameters related to body adiposity and insulin sensitivity were calculated. Results: Gene expression of pRb and p130 in human adipose tissues negatively correlated with BMI, percent body fat, fasting blood insulin levels and $HOMA_{IR}$. The correlations were absent or less obvious for p107. The adipose gene expression of pRb and p130 was reduced in the cafeteria diet-fed rats as compared to the controls rats, and increased upon reversion to a normal fat diet. Adipose p107 gene expression followed the opposite pattern: it was increased in the *obese* groups of animals (fed the high fat or the cafeteria diet), and it decreased upon reversion to a normal fat diet from a cafeteria diet. Conclusion: The results indicate that the expression of pocket proteins in adipose depots change in association with obesity. Both in rodents and humans, the mRNA expression of pRb and p130 is reduced in obesity, while that of p107 appears to follow a distinct direction.

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Programming of obesity susceptibility by different nutritional conditions during the perinatal period and its influence on insulin and leptin sensitivity in adulthood

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Nutritional conditions during gestation and/or lactation may affect the susceptibility to develop obesity in adulthood. Here, we aimed to assess whether early programming of obesity susceptibility may be related with differential programming effects on insulin and leptin sensitivity. For that purpose, particular nutritional conditions during gestation or lactation were studied in rats: a) 20% maternal caloric restriction during the first half of gestation, b) 30% maternal caloric restriction during lactation, and c) oral supplementation to neonate rats with physiological doses of leptin during lactation. Body weight and food intake were followed, and circulating levels of glucose, insulin and leptin were analysed under feeding and fasting conditions in adulthood. Results show that male and female offspring of caloric-restricted dams during gestation presented higher food intake than controls, accompanied by higher insulin levels and greater HOMA-IR index. In the same model, males displayed higher body weight and circulating leptin levels than controls. In contrast, both male and female offspring of calorie-restricted lactating dams showed lower body weight and ate fewer calories than controls; these rats were also protected against the increased insulin and leptin levels and HOMA-IR observed in controls when exposed to a high-fat diet. Finally, leptin-treated rats displayed lower body weight and food intake than controls, and also showed lower insulin and leptin levels and a decreased HOMA-IR. In conclusion, nutritional conditions during perinatal life affect insulin and leptin sensitivity, which may be related with the different propensity to obesity development in adulthood.

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Peripheral blood mononuclear cells reflect insensitivity to feeding conditions associated to obesity development

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Peripheral blood mononuclear cells (PBMC) constitute easy obtainable biological material with a great potential for nutrition and obesity studies. PBMC reflect nutritional response of key organs involved in energy homeostasis maintenance, which is altered in the obese state. We analyzed whether PBMC are able to reflect the insensitivity to changes in feeding conditions associated to obesity during the development of this pathology, in order to determine early markers of homeostatic disequilibrium. Expression of key genes central to energy metabolism was measured by Q-PCR in PBMC samples of normoweight (control) and cafeteria-fed rats (obese) in feeding/fasting/re-feeding conditions. PBMC samples were obtained monthly from 2 months (beginning of cafeteria diet-feeding) till 6 months of age. At this time-point gene expression was also analyzed in liver and adipose tissue samples. Our results show that PBMC reflect the response to feeding conditions characteristic of liver. In general terms, expression of genes related with fatty acid synthesis (*Fasn*, *Srebp1*) and adipogenesis (*Pparg*) decreases with fasting and increases with refeeding; while the expression of a key gene regulating beta-oxidation (*Cpt1a*) and the gene for an orexigenic neuropeptide (Npy), in accordance to their metabolic role, increase with fasting and decrease with refeeding. This expression pattern disappears in obese rats, in which insensitivity to feeding conditions is observed after only one month of cafeteria diet-feeding. We can conclude that during development, PBMC accurately reflect nutritional regulation of energy homeostasic genes and the insensitivity to feeding associated to obesity, even in the earlier stages with a low degree of overweight. Thus, these set of blood cells could constitute a potential source of biomarkers of early homeostatic imbalance useful in studies of nutrition that could help to predict/prevent the occurrence of overweight/obesity.

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CPT1A and SCL27A2 expression levels in blood cells as potential new biomarkers for the insulin resistant state associated with overweight in children

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Obesity and overweight in childhood have increased dramatically in last decades, so have the risks of associated medical conditions, such as type 2 diabetes and cardiovascular disease. Therefore, the identification of new early biomarkers of susceptibility is relevant to intervene with lifestyle and nutritional changes before the disease is manifested. Blood cells are useful as potential source of transcriptional biomarkers in human samples because they are easily accessible and changes in gene expression may reflect processes occurring at internal organs. Thus, the objective of this study was to evaluate, in blood samples of normal weight and overweight children, whether the expression levels of two genes involved in lipid metabolism, CPT1A and SLC27A2, are related with the insulin-resistant state associated to overweight. Blood samples were obtained from 306 normal-weight and overweight children, aged 2-9 years, from 8 different European countries. Whole-blood mRNA levels were assessed by RT-qPCR. CPT1A mRNA levels were overexpressed in males with overweight but not in females. In addition, we found a negative correlation between CPT1A mRNA levels and HOMA-index only in male children. Moreover, within overweight males, those with lower expression levels of this gene showed higher HOMA-index than those with higher expression levels. Concerning SLC27A2, the expression levels were lower in overweight compared with normal-weight children. Within overweight children, those with lower expression levels of this gene exhibited higher HOMA-index than those with higher expression levels, particularly males. It is concluded that CPT1A and SCL27 expression levels in blood cells appear to be good markers for the insulin resistant state associated with overweight/obesity in male children.

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Effects of a high fat diet feeding and grape seed procyanidin administration on plasma metabolome of hamsters

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The aim to this study was to evaluate the effect of the administration of a high fat diet (HFD) and a grape seed procyanidin extract (GSPE) in the plasma metabolomic profile of hamsters. For that purpose, two groups of hamsters were fed either a standard diet (STD) or a HFD for 15 days. At day 15, 8 animals of each group were sacrificed and the other animals were re-distributed in four groups (n=7-8) depending on the diet administrated (STD or HFD) and the treatment received (GSPE at 25 mg per kg of body weight/day or vehicle) from days 15 to 30 of the study. To detect the plasma metabolites, GC-MS and LC-TOF-MS were used and the data generated were analyzed with the XCMS software, which allows peak alignment and feature determination and identifies differences among treatments. Finally, LC-TQD-MS/MS was used to obtain the fragmentation pattern of the selected metabolites and tentatively confirm their identity. The XCMS analysis provided a list of 8 and 13 metabolites significantly changed ($p \leq 0.05$) as a consequence of the intake of the HFD during 15 and 30 days, respectively. The levels of most of these metabolites were higher in the HFD than in the STD-fed animals and are related with cholesterol and phospholipid metabolism (lysophosphatidylcholines and lysophosphatidylinositol). Six of these metabolites increased only in the HFD group at 15 days and could potentially be selected as early biomarkers of dyslipidemia. The GSPE supplementation modified 25 metabolites. Five of them increased in both STD and HFD GSPE treated groups, including oleamide, a fatty acid primary amide that exerts cannabimimetic effects and is involved in vasodilatation. In conclusion, the metabolomic analysis of plasma in hamsters could be a useful tool to detect early biomarkers of dyslipidemia and changes associated with the intake of GSPE.

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Early programming effects of moderate maternal caloric restriction during lactation on hepatic lipid metabolism capacity in offspring may be related with a better lipid handling in adulthood

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It is known that origins of obesity and its related metabolic alterations in adulthood lie not only in genetic heritability and environmental risk factors but are also associated to perinatal nutrition. Recent studies suggest that maternal nutritional during gestation and lactation may have important long-term effects on metabolic energy regulatory systems in offspring. We have described that the offspring of moderate calorie-restricted rats during lactation are protected from obesity and related metabolic pathologies in adulthood. Here we aimed to assess the early programming effects of this condition upon the hepatic expression profile of metabolic-related genes in the offspring and its relation with lipid-handling capacity and body weight gain in adulthood. Body weight and food intake of the offspring of control and 20% calorie-restricted dams during the suckling period (CR) were followed for 4m. At weaning (21d), some pups were killed and the liver was collected to perform gene expression analyses by RT-qPCR. At 4m, plasma triglyceride concentrations (TG) were determined at baseline and at different time-points after an oral load of olive oil (2.5 ml/Kg) (oral fat tolerance test). CR animals showed lower body weight and food intake than their controls during the whole follow-up. At weaning, CR animals presented higher hepatic mRNA levels of ATGL, CPT1 and ObRb versus their controls. No differences were found in TG levels between control and CR animals in adulthood, but the oral fat tolerance test revealed that CR animals (particularly males) showed a lower increase in TG after the oral load. In conclusion, moderate maternal caloric restriction during lactation results in early adaptations in the offspring, affecting adipogenic and oxidative capacity of the liver, in relation with a better fat handling, and their lower body weight gain in adulthood.

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Comparison of GIP release after oral glucose and fats loads in patients with metabolic syndrome (The Bioclaims Study)

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Introduction: Glucose-dependent insulinotropic polypeptide (GIP) is implicated in the pathogenesis of obesity and T2DM. The aim was to assess the differences in GIP release after glucose and fats loads in patients with metabolic syndrome (MS). Methods: Oral glucose tolerance test (OGTT) and oral lipids tolerance test (OLTT) were performed in MS (BMI= 30-40 kg/m^2) and controls (BMI<28 kg/m^2) aged 25-65 yrs. Blood concentrations of glucose, insulin, GIP were measured at fasting and every 30 min during OGTT and every 2hrs during OLTT. Results: In 36 MS patients (BMI= 34,6 kg/m^2 ± 3,84; glucose = 5,47 mmol/l) fasting GIP levels were 33,4 pg/ml ± 21,56 (OGTT) and 35,1 pg/ml ± 23,99 (OLTT). The highest concentrations during OGTT were observed at 30 min (190,8 pg/ml ± 80,97) and during OLTT at 2h (321 pg/ml ± 121,76). In 11 controls (BMI =27,5 kg/m^2 ± 0,98; glucose 5,29 mmol/l) fasting GIP were: 23,1 pg/ml ± 17,67 and 20,4 pg/ml ± 6,77 respectively. The most marked increase of GIP were observed at 30 min of OGTT (141 pg/ml ± 53,66) and 2h of OLTT (277,3 pg/ml ± 102,64). Fasting insulin levels were 18,38 µIU/ml ± 9,24 in MS and 10,07 µIU/ml ± 2,49 in controls. GIP and insulin levels were elevated in parallel in the postprandial state. Conclusion: MS patients revealed significantly higher GIP concentrations both fasting and postprandial compared to controls. Postprandial increase of GIP was higher during OLTT than OGTT.

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A core of plasma fatty acid could be gender-specific suitable markers to assess predisposition to obesity

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Introduction.- Early-life nutrition plays a significant role modulating metabolic homeostasis, including the potential susceptibility to obesity in adulthood. By feeding lactating dams with a leucine-supplemented diet (Leu), we have developed an animal model which results in a gender-specific susceptibility to adult obesity in the offspring. Leu males from offspring are slimmer than the corresponding controls under a high-fat diet in adulthood; in contrast, Leu females are more obese. Objective.- In this context, plasma fatty acids are known signaling molecules altered in obesity. The aim of this study was to assess their potential use as early-biomarkers of altered susceptibility to obesity. Methods.- Dams fed a standard chow diet (Leucine content 1.1%) received dietary leucine supplementation (final concentration 3.1% Leu) 24h after delivery until weaning (21 days). Then, offspring was fed a standard chow diet until adulthood. At 6 months of age, half of the animals (males and females) were fed a normal fat diet (10%kcal/fat) or a high-fat diet (45%kcal/fat) for 3 months. Plasma fatty acids were determined by gas chromatography. Results.- Offspring was non-obese at weaning however, a core of plasma fatty acids showed altered levels. Then, in adulthood under a high-fat diet, Leu-females showed a profile of fatty acids distinct from the one observed in the males. Therefore, a core of plasma fatty acids, involving pentadecanoic, arachidic, cis-vaccenic, hexadecenoic, oleic, gadoleic, á-linolenic, eicosatrienoic and docosahexaenoic acids could be gender-specific suitable markers to assess predisposition to obesity. Conclusion.- The comparison of the plasma fatty acid profile at weaning (in a non-obese status) with the one observed in adulthood, allows to define a set of biomarkers that could contribute to predict susceptibility to obesity in a gender specific manner.

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