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International Symposium on Immunonutrition 2017

Madrid, 17th–19th July, 2017

10th Anniversary

Abstracts

Guest Editor
A. Marcos, Madrid

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Immunonutrition has become, in relatively few years, an essential matter to discover and research on.

During the 19th mid-century, interactions between infections and malnutrition status, especially in children, were described by Simon. However, we needed one more century to establish the concept of this relationship, nowadays fully recognised due to the well-known review by Scrimshaw, Taylor and Gordon in the 1950s. After which there has been a wide expansion of the area, focusing not only on the consequences of malnutrition on the development of infection, but also on inflammatory processes: currently the heaviest flagstone of disease at any age. Indeed, during the last 20 years many chronic non-communicable diseases have appeared. This is the case of obesity which, in addition, is the trigger for the development of type 2 diabetes, cardiovascular and neurodegenerative diseases, as well as certain tumours. Moreover, obese people are more prone to suffer from more severe and frequent infections and allergies, than people with normal weight, probably due to premature aging, also known as the “inflammaging” process.

The first course on Immunonutrition was held in Madrid in 1994 and afterwards there has been a long list of international courses mainly held in Spain (1998, 1999, 2001, 2003, 2005 and 2007) and Latin America (Argentina-2003, Brazil-2004, Mexico-2006, Ecuador-2010, Mexico-2011, Venezuela-2012). In 2007 the i-FINER (international Forum for Immunonutrition Education and Research) group was created to support young researchers and exchange knowledge in this field among senior researchers and the first workshop was held in Valencia (Spain)-2007. Therefore, this event was planned in order to both update knowledge in the area and to encourage researchers to consider the inclusion of Immunonutrition in their scientific studies, taking into consideration the potential role of this interesting and relatively novel research field. It also gave new researchers and graduate students the opportunity to openly discuss their results with distinguished experts in the field. Thus, since 2007 a workshop has been annually organized in different countries (Argentina-2008, Spain-2009, Ecuador-2010, Mexico-2011, Spain-2012, Spain-2013, Italy-2014, Brazil-2015, and Mexico-2016). In between, the International Society for Immunonutrition (ISIN) was created in 2014 after the workshop in Brindisi (Italy) led by Drs. Emilio Jirillo and Mauro Serafini.

With this background, the ISIN leading group decided to celebrate the 10th Anniversary of these events in Madrid (Spain). For this purpose, 37 speakers from 14 countries participate in this Symposium. Their lectures are divided into 7 topic sections, such as: Exercise and the Immune System, Nutrition and Immune System Related Pathologies, Antioxidants and the Mediterranean Diet, Role of Micronutrients on the Immune System, Nutrigenetics and OMICS, Microbiota, Probiotics and Prebiotics I and II. In addition, two lectures are devoted to the methodology used in this field (Microbiota Assessment and Flow Cytometry). Moreover, two special lectures have been selected to include pressing topics related to inflammatory diseases and bioactive compounds. Finally,

both the opening and closing ceremonies are focused on the potential impact of global environmental change and its consequences on health and development of diseases with special attention to the deterioration of the immune system and the nutritional status.

This event has been organized by the CSIC (Consejo Superior de Investigaciones Científicas – Spanish National Research Council). The CSIC is the largest public research body in Spain. Located throughout the country, it plays an active role in scientific policy development in all the autonomous regions. On January 11th 1907, the Council for the Extension of Studies and Scientific Research [“Junta para Ampliación de Estudios e Investigaciones Científicas” (the JAE)] was created by the Ministry for Public Instruction and Fine Arts. In 1939, out of the JAE, the CSIC was created. As a multidisciplinary body, the CSIC covers all fields of knowledge (organised into eight scientific and technical areas), from basic research to advanced technological development.

In this special supplement in the Annals of Nutrition and Metabolism, 35 lecture abstracts plus 51 free abstracts presented at the Symposium on Immunonutrition, 10th Anniversary are included.

The International Society for Immunonutrition is indebted to several industries that have collaborated in the organization of this event, such as Bayer, Burson-Masteller, Coca-Cola, Danone, Heartgenetics, Sanofi, Vegenat, Ysonut with no involvement in the scientific affairs.

I personally would like to acknowledge the involvement of my research group (Esther Nova, Sonia Gómez-Martínez, Ligia-Esperanza Díaz, Noemí Redondo) for their constant support working very hard at the lab during the whole time I have dedicated to the organization of this event. Without them, I would not have been able to do it.

Finally, I appreciate very much the involvement of the scientific and organizing committees to share with me the enthusiasm to disseminate the knowledge of the interesting research area of Immunonutrition.

Ascensión Marcos

On behalf of the ISIN Board

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The Importance of a Balanced Omega-6 to Omega-3 Ratio in the Prevention and Management of Obesity

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In the past three decades, total fat and saturated fat intake as a percentage of total calories has continuously decreased in Western diets, while the intake of omega-6 fatty acid increased and the omega-3 fatty acid decreased, resulting in a large increase in the omega-6/omega-3 ratio from 1:1 during evolution to 20:1 today or even higher. This change in the composition of fatty acids parallels a significant increase in the prevalence of overweight and obesity. Experimental studies have suggested that omega-6 and omega-3 fatty acids elicit divergent effects on body fat gain through mechanisms of adipogenesis, browning of adipose tissue, lipid homeostasis, brain-gut adipose tissue axis, and most importantly systemic inflammation. Prospective studies clearly show an increase in the risk of obesity as the level of omega-6 fatty acids and the omega-6/omega-3 ratio increase in red blood cell (RBC) membrane phospholipids, whereas high omega-3 RBC membrane phospholipids decrease the risk of obesity. Recent studies in humans show that in addition to absolute amounts of omega-6 and omega-3 fatty acid intake, the omega-6/omega-3 ratio plays an important role in increasing the development of obesity via both AA eicosanoid metabolites and hyperactivity of the cannabinoid system, which can be reversed with increased intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). A balanced omega 6/omega-3 ratio is important for health and in the prevention and management of obesity.

Keywords: Obesity, Balanced omega-6/omega-3 fatty acid ratio, Inflammation, Endocannabinoids, Browning of adipose tissue.

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High Fat Meal as Inducers of Postprandial Cellular Stress: The Red-Ox Role of Probiotics

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Oxidative and inflammatory stress, arising from sustained postprandial hyperlipidemia and hyperglycemia, are associated with a higher risk for CVD and obesity. In western societies where most of the day is spent in the postprandial state, postprandial stress a key variable involved in the development of cardiovascular

risk factors. The inflammatory response after High Fat Meal (HFM) is mediated by pro-inflammatory cytokines, glycemia/insulin response and oxidized lipids. In overweight subjects, the metabolic stress induced by HFM trigger an endogenous antioxidants response, increasing uric acid and thiols groups, to counteract the onset of postprandial inflammatory/oxidative meals.

A large body of evidence have been provided on the health effects of probiotics, suggested to be link to a down-regulation of the production of inflammatory cytokines and an enhanced synthesis of IL-4 and IL-10 by CD4+ T cells. Moreover, several results suggest that consumption of *L. casei* Shirota-fermented milk is able to modulate the composition and metabolic activity of the intestinal microbiota. In this view, novel evidences suggests that dietary flavonoids not absorbed in the small intestine are transformed by the colonic micro-flora to bioactive and functional metabolites, potentially able to play a role in the modulation of oxidative and inflammatory stress by plant foods.

We hypothesize that probiotics might play a role in mitigating oxidative and inflammatory stress induced by unbalanced energy meals through a modulation of endogenous antioxidant and anti-inflammatory defences and contributing to the production of bioactive metabolites of flavonoids from microbiota.

Keywords: Probiotics, antioxidant, post-prandial stress, inflammation, Caco2 cells.

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Fatty Acids Interactions with the Nlrp3 Inflammasome and IL-1 β biology

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Nutrition is an important environmental factor which can modulate metabolic-inflammation. Recent research has highlighted the importance of Nlrp3 mediated IL- β activation on insulin signalling within the context of obesity and type 2 diabetes (T2D) risk. Conversely recent work shows that metabolism is a key determinant of inflammation. Thus metabolic-inflammation is a key biological interaction; however there is a paucity of data in relation to the nature and the extent to which nutritional status affects metabolic-inflammation. Our work has demonstrated that different fatty acids differentially modulate metabolic-inflammation. This paradigm will be discussed within the context of if/how dietary fatty acid composition may modulate obesity and T2D risk, via chronic inflammatory and metabolic processes.

Acknowledgments: This work was largely completed by Claire Lyons and Orla Finucane.

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ance 'FOOTBALL' (14/JP-HDHL/B3076); Enterprise Ireland funded Food for Health Ireland (FHI) programme (TC2013-0001) and the Irish Department of Agriculture, Food and the Marine, 'ImmunoMet – dietary manipulation of microbiota diversity for controlling immune function' programme (14/F/828).

Keywords: Nlrp3 inflammasome, dietary fatty acids, metabolic-inflammation.

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The Human Milk Microbiota

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Human milk has been traditionally considered sterile; however, recent studies have shown that it represents a continuous supply of commensal, mutualistic and/or potentially probiotic bacteria to the infant gut. Culture-dependent and -independent techniques have revealed the dominance of staphylococci, streptococci, lactic acid bacteria and bifidobacteria in this biological fluid, and their role on the colonization of the infant gut. These bacteria could protect the infant against infections and contribute to the maturation of the immune system, among other functions. Different studies suggest that some bacteria present in the maternal gut could reach the mammary gland during late pregnancy and lactation through a mechanism involving gut monocytes. Thus, modulation of maternal gut microbiota during pregnancy and lactation could have a direct effect on infant health. On the other hand, mammary dysbiosis may lead to mastitis, a condition that represents the first medical cause for undesired weaning. Selected strains isolated from milk can be good candidates for use as probiotics.

Keywords: Human milk, microbiota, microbiome, mastitis, lactation.

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Probiotics in Pediatrics

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Increasing importance is given to the modulation of the intestinal microbiota by the use of probiotics, prebiotics and symbiotics to treat several diseases, mainly gastrointestinal problems, such as different types of diarrhea (from the community, associated with antibiotics, traveler's diarrhea, intolerance lactose, etc.), functional disorders (infant colic or irritable bowel syndrome) or inflammatory processes (ulcerative colitis). They are also used successfully in various diseases of the woman (vulvovaginitis and mastitis) and its effect on allergies such as atopic dermatitis and the prevention of infections (from premature neonate to old person) has been evaluated.

Research in experimental animals and in humans opens the door to future applications with the supplementation of these organisms and nutrients. There are studies that would support its use in nutritional problems (obesity, malnutrition), neurological diseases and behavioral alterations (autism, depression, anxiety), in periodontal disease and eventual prevention in several types of cancer. Finally, there is progress in the repercussions that the alteration of the microbiota can have on the appearance of cardiovascular diseases, arteriosclerosis, diabetes, hypercholesterolemia, metabolic syndrome, etc.

Pediatrics is one of the medical speciality where the meta-analyses and systematic reviews of contrasted clinical trials have demonstrated their efficacy in several pathologies, especially digestive ones, reason why their use is gradually establishing itself in the habitual clinical practice of the paediatricians. In addition there are several lines of research open in probiotic supplementation in childhood.

Keywords: Probiotics, microbiota, children.

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Impact of Anorexia Nervosa on Immunological Biomarkers

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Anorexia Nervosa (AN) affects all systems in the body and evidences have shown that multiple adaptive mechanisms are triggered under this metabolically and psychologically demanding

situation which also involves the immune system. Leukopenia and relative lymphocytosis are among the most frequent abnormalities. In our follow-up study on AN patients, a group of 66 females, aged 12–17 years, referred for their first episode of either AN or Feeding or Eating Disorders Not Elsewhere Classified (FED-NEC) were studied upon admission (46 AN-restricting subtype, 11 AN-binge/purging subtype and 9 FED-NEC). Relative lymphocytosis was negatively correlated with Z-score BMI. In regression models, leptin explained even more variability in the lymphocyte percentage than BMI, suggesting that this is a leptin-mediated process. Furthermore, weight loss induced an increase of naïve CD4+ lymphocytes suggesting a priority to preserve adaptive immunity. Forty-one of the patients participated in a case-control study, matched by sex, age and socioeconomic status. The low leptin and increased adiponectin levels found in patients are in concordance with findings in the literature, and so are the high basal cortisol levels. However, the elevation of pro-inflammatory cytokines was not confirmed in these young patients with early diagnosis. One-year follow-up of blood biomarkers showed partial leptin recovery in parallel to BMI increase, while adiponectin remained high. There seems to be a delayed response of adiponectin to nutritional changes. Finally, inconsistencies are observed in the relationships between basal biomarker alterations and the severity of eating disorder psychopathology. Studying how these relationships change along with treatment should provide clues regarding the link between psychological factors and metabolic adjustments.

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Keywords: Anorexia nervosa, Lymphocyte subsets, adipokines.

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Relationships Between Fat Composition and Inflammatory Markers in Subjects with Metabolic Syndrome (MS) Features

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Background and Aim: Adipose tissue has important endocrine functions by producing various hormones and signaling molecules. Some substances secreted by the adipose tissue are actively involved in the regulation of immune function and inflammatory processes. The aim of this study was to evaluate the influence of a dietary strategy for weight loss (the RESMENA [reduction of metabolic syndrome in Navarra. Spain.] diet) on inflammation markers from individuals with metabolic syndrome (MS).

Methods: Seventy-five participants (51.6% men; 49 ± 9 years; BMI: 32.8 ± 20 kg/m²) of RESMENA study were evaluated at baseline and after 8 weeks. Anthropometric, body composition (DXA), glycemic and lipid profile (Pentra C-200) as well as inflammatory markers (specific kits) were determined.

Results: A Mediterranean-based nutritional intervention was able to induce beneficial changes in body weight, waist circumference, body fat and visceral adipose tissue (VAT) as well as on glucose and lipid profiles. Interestingly, adiponectin and the ratio adiponectin/leptin (A/L) significantly increased after two months of follow-up. Thus, a linear regression model was set up to assess the influence of changes in VAT on changes in A/L ($\beta = 0.082$; $R^2 = 0.102$; $p = 0.041$) revealing that the association partly depend on adipose tissue.

Conclusion: Available data evidence an inverse relationship between inflammation and visceral adipose tissue, which envisages the important role of weight management and immunonutrition/anti-inflammatory strategies to explain and treat metabolic syndrome features within precision nutrition approaches.

Acknowledgments: The authors are grateful to the volunteers of the study as well as to the physician Blanca E. Martínez de Morentín, the nurse Salomé Pérez, the technician Verónica Ciauriz and the dietitians Rocio de la Iglesia, Patricia Lopez-Legarrea and Aurora Pérez-Cornago for the contribution to RESMENA project. The pre-doctoral research grant to Irene Cantero from the Centre for Nutrition Research of the University of Navarra is gratefully acknowledged. Finally, we want to thank to the Health Department of the Government of Navarra (48/2009; 61/2015), The Linea Especial about Nutrition, Obesity and Health (University of Navarra LE/97), CIBERObn (Physiopathology of Obesity and Nutrition) and RETICS for their support.

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Keywords: Immunonutrition, weight loss, adipose tissue, metabolic syndrome, RESMENA.

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How Prebiotics Can Help Probiotics

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The regular intake of dietary fibre has been found to favour the diversity of gut bacteria. Prebiotics are selectively fermented non-digestible food ingredients that specifically support the growth and/or activity of health-promoting bacteria that colonize the gastrointestinal tract. In this perspective, prebiotics usually favours bifidobacteria and lactobacilli growth. Synbiotics refer to food ingredients or dietary supplements combining probiotics and prebiotics. There are prebiotics having strong scientific evidence of their beneficial properties in humans such as inulin-type fructans, galacto-oligosaccharides, lactulose and human milk oligosaccharides. Research studies in prebiotics have demonstrated their beneficial effects on functional constipation and energetic metabolism, including reduction of post-prandial glycaemic responses and satiety. In vitro and in vivo models and intervention trials in humans are needed to evaluate the health benefits of prebiotics. Perspectives in studying prebiotics are focused on the evaluation of the effects of prebiotics in the gut microbiome, the description of mechanisms that contribute to health benefits, and the unravelling of direct prebiotics benefits that are not necessarily related to microbiota modulation, such as antimicrobial and immunological effects.

Financial Support: Research projects AGL2012-35814, AGL2016-75951-R and P916PTE0233.

Keywords: Prebiotics, Probiotics, Synbiotics, Health benefits.

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Role of Probiotics in the Aging Process

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During the aging process, which starts at the adult age and finishes with the death of the individual, all the physiological systems begin to be impaired, but especially those charged with the maintenance of homeostasis such as the nervous, endocrine and immune systems. Thus, with aging the risk of suffering diseases increases. A healthy longevity depends about 75% on lifestyle factors, and nutrition is one of the factors which can most affect the aging process. Since the principal cause of aging is the oxidative and inflammatory stress of the organism, in which the immune system has a relevant role, the ingestion of diets with adequate amounts of antioxidants has been shown effective for improving the redox state and the immune function and consequently in reaching a healthy longevity. Probiotics, which are live microorganisms that provide health benefits when consumed, are capable of enhancing many immune and nervous capacities. Although in aging these effects have scarcely been studied, the ingestion of several strains of *Lactobacillus* and *Bifidobacterium* seems to be useful. This re-

search on the effect of probiotics on aging has been focused on their capacity to restore the microbiota, which are altered with age, as well as on their effectiveness in reducing several symptoms of diseases, which are frequent with age. Moreover, probiotics improve the homeostatic system functions and consequently increase longevity. This effect is mediated through their antioxidant and anti-inflammatory capacities. In conclusion, probiotics can be good candidates to improve immunity and achieve a healthy longevity.

Financial Support: This work was supported by the Health Institute Carlos III and the European Regional Development Fund (PI15/01787).

Keywords: Aging. Probiotics. Microbiota. Immune system. Nervous system.

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Consensus Statements on Immunonutrition and Exercise

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Close interactions between diet and the immune system are the genesis of the term “Immunonutrition”. Many articles have been published in the field of immunonutrition since the mid-1940s. The topic has more recently become of interest to those working on exercise immunology, leading to approximately 400 publications. Of these, about 130 papers on immunonutrition and exercise have been published in the last 3 years, making this a rapidly growing area of research.

Stressors such as high intensity or exhausting training programmes, sleep disturbances, and long-haul flights are potential immune disruptors sometimes leading to immunodepression and increased likelihood of illness. In order to minimise these phenomena and to optimise recovery, nutritional interventions are often considered by athletes as possible countermeasures to exercise-induced immunodepression. However, only a few nutrients have so far shown any positive effects in maintaining the immune health of athletes.

Elderly and overweight or obese individuals are often prescribed physical training programmes as a countermeasure to immunodepression (“Exercise as Medicine”). In these populations, nutrition adds the possibility of additional valuable support.

Our consensus statements on immunonutrition and exercise (Berman et al., 2017, *Ex Immunol Rev*) include contributions from researchers worldwide. They provide a consensus: on updated science, the aspects for which a consensus actually exists, together with the controversies. Topics, some of which will be addressed in the talk, include carbohydrates; fatty acids; amino acids; polyphenols and herbal supplements; antioxidants; minerals; pro- and prebiotics; vitamin D; and bovine colostrum. There are also sections on: Immunonutrition in the exercising obese and overweight, and in elderly individuals; and on biomarkers; concluding with a comprehensive table on relevant investigative methods.

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Milk Fat Globule Membrane as a Diet Source of Bioactive Phospholipids and Sphingolipids and their Impact in Human Health

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Milk lipids, are present in the form of dispersed fat globules enveloped by a complex interfacial phospholipid membrane known as the milk fat globule membrane (MFGM). Besides glycerophospholipids (PL), MFGM contains sphingomyelin (SM), glucosyl- and lactosylceramides, and gangliosides. Those complex lipids have been shown to participate in a variety of indispensable metabolic, neurological, and intracellular signalling processes such as development necrosis and apoptosis, transport, DNA replication, neuronal signaling, or secretion, to name a few. Also it have been reported that MFGM may prevent the mild cognitive decline and neurodegeneration as associated with age, and improve the physical performance of healthy middle-aged adults. Supplementation of MFGM in infants and in children may decrease the risk of infections, with glycosylated proteins and membrane lipids being the most likely candidates for this protective effect, acting as a decoy for pathogens and viruses. Significant advances have been made in understanding the technological, functional and nutritional benefits of the MFGM, however, much more remains to be discovered.

Financial Support: This work was financially supported by the Spanish Ministry of Science and Innovation and European FEDER Funds from projects AGL 2014-56464.

Keywords: Buttermilk; polar lipids; phospholipids; sphingolipids.

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Usefulness of the Microbiota Assessment in Immunonutrition

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Symbiotic relationships between human body and foreign organisms have evolved for long, so that a number of our vital functions are actually performed by non-human cells. Intestinal flora is a major driving force which helps shaping our immune system and keeping us healthy, affecting metabolic, endocrine, immune and even neural activity.

Novel sequencing technologies are very well suited to investigate the diversity and population of commensal flora, based on three major characteristics: i) an enormous productivity which allows analysis of millions of molecules at an incredibly low cost; ii) the omission of cloning steps during lab workflows, which accelerates the whole process and avoids cloning bias; and iii) the indi-

vidualized analysis of single molecules, giving visibility to minority but possibly relevant populations.

Much of the work is focused on taxonomic analysis, where 16S-based sequencing is able to give an in-depth view of the diversity of bacterial populations, at least up to the genus level, and the modification of bacterial “profiles” associated to diets, disease, ageing, etc. However, sequencing technologies can also be applied to whole metagenome analysis in search of new genes or variants that may provide specific abilities, or to meta-transcriptomes, in order to find bacterial or viral genes which are switched on and off in the very different physiological or pathological situations.

Combined with metabolomics’ and proteomics’ data, metagenomics studies can provide a very deep and comprehensive view of the role and the mechanisms used by our microbiota in the complex equilibrium between health and disease.

Keywords: Genetic tools, microbiota assessment.

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Biochemical Nutritional Profile in Specific Pathologies

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Introduction: Malnutrition status in patients suffering from colorectal, esophageal or stomach cancer has been scarcely studied. Inadequate nutrient intake is probably one of the main factors involved.

Objective: To evaluate the nutritional status of patients at the beginning of the specific treatment by using anthropometric and biochemical parameters.

Material and Methods: Body weight (w, kg), height (h, m) and Body Mass Index (BMI) (w/h^2 , kg/m^2) were determined in 32 adult patients who attended the Service of Gastroenterology at Carlos G. Durand Hospital, Buenos Aires, Argentina. Blood samples were collected from fasting patients. Specific plasma protein fractions were potentially useful for the nutritional evaluation [Albumin, Transthyretin (TTR), Transferrin, C3c, C4c and Apo lipoprotein B (ApoB)] were measured by single radial immunodiffusion technique on agar gel layers (Diffu-Plate Biocientífica S.A., Argentina and Binding Site, UK).

Results: We observed a statistically significant decrease in plasma concentration of Transferrin, ApoB, C4c and TTR when data were compared with reference values (Dunnett’s test). These findings suggest nutritional disorders in the group of patients, with an increase in plasma albumin which points out a haemoconcentration status. However, the BMI level was compatible with an adequate nutritional status.

Conclusions: The biochemical markers showed differences between patients with different types of cancer; patients suffering from esophageal or stomach cancer seemed to show a much deeper depleted nutritional status than the rest of patients.

Acknowledgments: Octavio Casbarien MD, for selection of patients. Elise Fabios MD, for technical assistance.

Financial Support: University of Buenos Aires, Argentina.

Keywords: Colorectal cancer – esophageal cancer – stomach cancer – nutritional evaluation – biochemical parameters.

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Are You Eating Right? Ask Your Genes: Developing a Value Proposition in Nutrigenetics

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Genetic testing, a major promise to foster personalized healthcare for over a decade, has frequently failed to deliver a solution suitable for a global usage of its claimed advantages. The major barrier to its success is commonly known to be the gap between getting the genetic data and providing actionable advice.

Recent trends in genetic testing are showing strong signals that the adoption curve is about to grow significantly [1] with the adoption of genetic tests in several health fields, including nutrition and fitness. Nowadays, more than 2,000 genetic tests are commercially available, not only in healthcare facilities but also online [2, 3].

The genetic testing market is the fastest growing segment in molecular diagnosis [4]. In this market, finding the product value is key for companies to meet the customer expectation [5]. Within genetic healthcare advice, 80% of consumers look firstly for usefulness, privacy protection, and cost-effectiveness. Besides the perceived personalized healthcare advantage, consumers also take into consideration the scientific validity, the effectiveness of recommended actions, the accessibility to the product and the possibility of coaching or further advice [6].

HeartGenetics (www.heartgenetics.com) developed a new certified nutrigenetic product, which consists on a genetic test (MyNutriGenes[®]) associated with machine-learning algorithms, that is the most informative and targeted product in the market, to be used for the definition of a personalized nutritional plan. Based on the most relevant and statistically significant genotype-phenotype studies, this non-direct to consumer and cost-effective product was successfully validated in more than 1000 cases.

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Conflict of Interest: Maria Raimundo, Susana Santos and Ana Teresa Freitas are affiliated with HeartGenetics, a Portuguese start-up company that develops genetic tests and software tools to be used on human genetics.

Keywords: Nutrigenetics; Value proposition; MyNutriGenes; Genetic test.

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Probiotic Adaptation to the Gut Environment and Interaction with the Host

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Bifidobacteria and lactobacilli are natural inhabitants of the human gastrointestinal tract and some strains are able to exert health-promoting effects, being considered as probiotics. The scarcity of molecular tools has hindered the identification of mechanisms underlying their behavior, as well as their interactions with the host. Some of these experimental obstacles have been successfully overcome with the use of omics technologies. The aim of this presentation is to summarize some of the work related to the adaptation abilities of lactobacilli and bifidobacteria to the gut environment, mainly focused on the study of the response of probiotics to gastrointestinal conditions and their interactions with human immune cells and enterocytes, with a special focus on bifidobacteria.

Proteomics, transcriptomics and functional genomics have been used for a deeper understanding of probiotic mechanisms. Bile and acid pH represent a key challenge for bifidobacterial survival in the gastrointestinal environment and may, thus, determine their functionality at this location. Acid response involves a number of changes jointly dedicated to mitigate the impact of protons in the cell physiology, and exposure to bile produces deep changes in the carbohydrate metabolism, as well as in fatty acid biosynthesis and the activity of bile-efflux proteins. It is also worth mentioning that bile regulates the local concentration of some bifidobacterial adhesins. On the other hand, we have characterized the re-

sponse of different immune cells and enterocytes to a variety of strains, showing that *B. bifidum* displays a particular immunomodulatory activity and plays a key role in T cell reprogramming.

Keywords: bifidobacteria, lactobacilli, probiotics, adaptation, cross-talk.

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Iron Deficiency Indicators and Immune Response

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Increasing obesity and iron-deficiency anemia (ID) are major global public health problems. Even though they represent opposite ends of nutritional situations, overweight individuals are at higher risk of iron deficiency than normal-weight individuals. Iron is required by the host to mount an effective immune response, and evidence suggests that there is an increased risk of infections during ID. Serum ferritin, iron and total iron binding capacity, erythrocyte protoporphyrin, hemoglobin, hematocrit, mean red cell volume and red blood cell distribution width. can be used individually or in combination to assess the status of an individual's iron stores. Hemoglobin, hematocrit and erythrocyte protoporphyrin assessments require small amount of blood but they are functionally late indicators of iron deficiency and can be affected by infection, inflammation, acute phase reactants or illnesses. Cytokines are important mediators of cellular immune activity, with the micro-environmental cytokine profile determining the balance between, and the intensity of, cellular and humoral immune responsiveness. Little is known concerning the effects of iron deficiency on cytokines. Iron deficiency is common in obese people and a state of subclinical inflammation that arrest iron in the reticuloendothelial system by releasing different mediators has been implied. Obesity-related inflammation may play a central role through its regulation of hepcidin, due to its increase that may reduce iron absorption and blunt the effects of iron fortification. Low iron status in obesity may result from a combination of nutritional and functional iron deficiency. Subclinical inflammation in obesity, and its effect on hepcidin levels, are reviewed as the possible explanation for the phenomenon.

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Flavonoids as Modulators of Microbiota and Its Cross-Talk with the Immune System: The Cocoa Case

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Intestinal microbiota interacts with the host cells by a process known as crosstalk. This process occurs, among other mechanisms, through the interaction of certain molecules of the microorganisms with the toll-like receptors (TLRs) present in the intestinal cells, both epithelial and immune cells. These pattern-recognition receptors are then involved in the first line of defence against pathogens and play a key role in both innate and adaptive immunity. Microbial disbiosis, and also dysregulation in this type of bacteria-host interaction, are involved in the development of chronic and severe inflammation as well as immunological disorders.

Different types of flavonoids have demonstrated their ability to modify microbial composition, some of them counteracting pathogen growth but, some others acting as some specific bacterial growth promoters. Moreover, there is growing evidence that they can also modulate the TLR-mediated signalling by interfering at several steps of the pathways involved: they can modulate TLR gene and protein expression, and they can also regulate the downstream signalling molecules involved in the TLR pathway. These synergistic mechanisms suggest the role of some flavonoids in the preventive effect on certain chronic diseases.

Cocoa is one of the foods rich in flavonoids, although some other bioactive components co-exist, which has demonstrated changes in microbiota as well as modification of TLR-mediated signalling.

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Keywords: TLR; polyphenols; inflammation; vegetables; intracellular signalling.

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Vitamin D and Immunity in Type 2 Diabetes Mellitus

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Introduction: Pre-vitamin D is synthesized in skin from 7-dehydrocholesterol by exposure to UV light, transported by its binding protein (DBP) and bioconverted into 25-hydroxyvitamin D in liver, although the intestine and kidney may also convert it. It is known to regulate calcium and phosphorous homeostasis, cellular differentiation and proliferation, as well as parathyroid function.

Objective: To review information on the effects of vitamin D or its deficiency, on the immune system in relation to type 2 diabetes mellitus (T2DM).

Results: Vitamin D receptors are found in almost every type of cell, and even though its most well known functions are related to bone health, vitamin D is extremely important for other metabolic activities. In the immune system, it promotes activation of the Th1 pathway for innate immunity and the Th2 pathway for anti-inflammatory cytokine production and suppression of B-lymphocytes. Vitamin D is involved in glucose homeostasis, and T2DM; vitamin D may influence insulin secretion and sensitivity. We have observed diverse effects of vitamin D supplementation in T2DM on cytokine and adipokine production, with maintenance of glucose homeostasis.

Key Findings: The immune system is partially modulated by vitamin D and this may be affecting the development of T2DM as it has important immune system components.

Conclusions: It is necessary to undertake more research in relation to the effect of vitamin D and its deficiency on the immune system of patients with type 2 diabetes mellitus, at different vitamin D intake and sun exposure levels.

Keywords: Vitamin D, Immune System, type 2 Diabetes Mellitus.

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Omega-3 Fatty Acids and Inflammatory Processes

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Inflammation is a condition which contributes to a range of human diseases. It involves a multitude of cell types, chemical mediators, and interactions. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are omega-3 (n-3) fatty acids found in oily fish and fish oil supplements. These fatty acids are able to partly inhibit a number of aspects of inflammation including leucocyte chemotaxis, adhesion molecule expression and leucocyte-endothelial adhesive interactions, production of eicosanoids like prostaglandins and leukotrienes from the n-6 fatty acid arachidonic acid, and production of inflammatory cytokines. In addition, EPA gives rise to eicosanoids that often have lower biological potency than those produced from arachidonic acid and EPA and DHA give rise to anti-inflammatory and inflammation resolving mediators called resolvins, protectins and maresins. Mechanisms underlying the anti-inflammatory actions of marine n-3 fatty acids include altered cell membrane phospholipid fatty acid composition, disruption of lipid rafts, inhibition of activation of the pro-inflammatory transcription factor nuclear factor kappa B so reducing expression of inflammatory genes, activation of the anti-inflammatory transcription factor peroxisome proliferator activated receptor γ and binding to the G protein coupled receptor GPR120. These mechanisms are interlinked, although the full extent of this is not yet elucidated. Animal experiments demonstrate benefit from marine n-3 fatty acids in a range of models of inflammatory conditions. Human trials demonstrate benefit of oral n-3 fatty acids in some inflammatory diseases. There is growing interest in whether these effects of marine n-3 fatty acids may be useful to control chronic low-grade inflammation.

Keywords: Omega-3; Inflammation; Eicosanoid; Cytokine.

Exercise and the Immune System: Physical Activity as a Therapy

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Exercise modulates all aspects of the immune function, but it is particularly important in modulating the innate/inflammatory response. Currently, physical activity is frequently being applied in the prevention and treatment not only of infectious and inflammatory diseases, but also in pathologies with an underlying impaired functioning of the immune system, particularly in chronic low-grade inflammatory pathologies. Nevertheless, it is crucial to separate the preventive and the therapeutic effects of exercise. Conceptually, as a purely preventive strategy, exercise should be recommended for the healthy population. As a therapeutic intervention, however, given that the individuals are not healthy, special atten-

tion must be paid to the type of exercise (intensity, duration, and modality) and the timing of its prescription for each type of pathology and to other co-existing disorders. In addition, the biomedical side effects of exercise also need to be considered.

Concepts and conclusions related to how exercise affects the innate and/or inflammatory responses mediated via immune-neuroendocrine interactions are revised and analyzed. The concepts of “the bioregulatory effect of exercise” [1], exercise as a “polypill” [2], and “dangerous exercise” by eliciting danger-stress and inflammatory responses [3] will be discussed. Even in metabolic syndrome, for which the benefit of regular exercise is clearly accepted, there have been reports of “dangerous exercise”. As an example, recent results on the effect of regular exercise-induced stress without diet in the obese Zucker rat model of the metabolic syndrome are also discussed.

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Keywords: Exercise, stress, inflammation, immune system, metabolic syndrome.

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Immune Mechanisms Mediated by Probiotic Bacteria

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The gastrointestinal tract is an ecosystems containing microorganisms crucial for the maturation of the immune cells. Probiotic microorganisms induce immune activation through: a) stimulation the epithelial cells (IEC), by adhesion or by their cell wall fragments b) activation of immune cells of the gut c) induce antimicrobial substances d) probiotic interaction with IEC, produce cytokines and chemokines to stimulate the Mucosal Immune System (MIS). We demonstrate: 1) Probiotic bacteria or their fragments interact with the IEC, 2) this activation stimulate the MIS increasing the IgA cells in the intestine, bronchus and breast and inducing immunomodulation through the cytokine IL10. 3) the IEC induces cytokines as IL6, MCP1 favoring the expansion of LB, the IgA +cells and infiltration of mononuclear cells as macrophages, to produce IL10 and maintain homeostasis.4) The interaction with the IEC, TLRs are involved, and CD206 (mannose receptor) in macrophages and dendritic cells 5) probiotics do not induce in-

flammatory immune response, only produce a slight increase in the immune cells of the lamina propria 6) The main immune response induced by probiotics is the Innate response; 7) There is not negative effect by the prolonged consumption 8) probiotics in allergy induce Th1 balance with production of IgG not IgE; 9) they induce an increase in the functionality and microbicidal activity in distant cells from the gut as peritoneal and spleen macrophages.

Keywords: Probiotics, Mechanisms, Mucosal Immune System.

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Diabetes. Is It Possible to Avoid Drugs by Changing Diet?

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Background and Objectives: The effect of n-3 fatty acids on glycemic control in Chinese type 2 diabetic (T2D) patients is not clear. The aim of the present study was to investigate the effects of n-3 fatty acid supplements, both marine and plant based, on glycemic traits in Chinese T2D patients.

Methods: In a double-blind randomized controlled trial, 185 recruited Chinese T2D patients were randomized to either fish oil (FO, n = 63), flaxseed oil (FSO, n = 61) or corn oil group (CO, served as control group, n = 61) for 180 days. The patients were asked to take corresponding oil capsules (4 capsules/day, 1 g/capsule), which totally provided 2 g/day of eicosapentaenoic acid + docosahexaenoic acid in FO group and 2.5 g/day of alpha-linolenic acid in FSO group. We have measured the blood concentration of glycated haemoglobin (HbA1c), insulin and glucose, and we also calculated homeostatic model assessment of insulin resistance (HOMA-IR).

Results: No significant group×time interaction was observed for HOMA-IR, fasting insulin or glucose. Significant interaction (P = 0.035) between the study groups (3 groups) and time (3 time points) for HbA1c was observed, with HbA1c decreased significantly in FO group compared with CO group (P = 0.037). We also found significant group×time interactions for low-density lipoprotein cholesterol (LDL-C) (P = 0.043), total cholesterol (TC) (P = 0.021), total cholesterol/high-density lipoprotein cholesterol (TC/HDL-C) (P = 0.009) and triacylglycerol (TG) (P = 0.003). Post-hoc analyses indicated that serum concentrations of TC (P = 0.029), TC/HDL-C (P = 0.038) and TG (P = 0.001) were decreased significantly in FO group compared with CO group.

Conclusions: Marine n-3 PUFA supplements may improve glycemic control and lipid profiles among Chinese type 2 diabetic patients.

Keywords: n-3 PUFA, type 2 diabetes, fish oil, flaxseed oil, corn oil.

Applicability of Flow Cytometry to the Study of the Nutritional Status

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Flow cytometry is the key tool for immunology research. Therefore, is the standard technique to analyze the immune status. By using different fluorochromes attached to specific antibodies, several parameters can be detected simultaneously, allowing to work with a small amount of sample. Typical analysis are Immunophenotyping (PBMCs, lymph nodes, spleen populations, etc.), functionality (T cell activation, phagocytosis, oxidative burst, proliferation, etc.), Immune Regulation (cytokines, soluble cytokine receptors, chemokines, immunoglobulins, acute-phase proteins, etc.), total lymphocyte or lymphocyte subpopulations counts, detection of rare regulatory immune cells (Tregs, memory or naïve cells, etc.) or even other kind of rare cells like circulating lipid-rich cells.

Flow cytometry technology allows, not only multiparameter analysis of complex populations, but the effective isolation of subpopulation or subpopulations of interest (up to six), in order to culture them or to process for Nucleic Acid Sequencing or perform other analysis.

A side application of Flow Cytometry in the field of Immunonutrition is the detection of microbial contamination in foods and beverages and how these contaminants affect microbiota and immune status. Also the analysis of interactions in complex populations of microorganisms (like mouth or gut microbiota) can be accomplished using flow cytometry.

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Authorship: Alberto Álvarez-Barrientos, Pilar Torralbo Jiménez y Yolanda Gutiérrez Martín.

Keywords: Immunophenotyping, Functionality, Immune Regulation, sorting.

Reference

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Nutritional Solutions to Reduce Risks of Ill Health Due to Air Pollution

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Around 85% of the global population lives in regions where air pollution levels exceed the air quality guideline threshold established by World Health Organization. Among other pollutants,

exposure to particulate matter (i.e. PM_{2.5}) has been associated with increased cardiovascular and respiratory morbidity and mortality. In 2015, PM_{2.5} pollution contributed to 4.2 million deaths worldwide, and was the 5th global risk factor for mortality. While national and international regulatory and environmental health organizations are advocating to reduce human exposure to air pollution, there may be related nutritional opportunities to diminish individual susceptibility as well. Emerging evidence suggests that the most effective health interventions likely operate along common inflammatory and oxidative stress pathways impacted by various gaseous and particulate pollutants. During the last two decades, several human clinical studies have suggested that the negative health impacts of some air pollutants may be altered by intake of essential micronutrients (e.g. vitamins), and long-chain polyunsaturated fatty acids with anti-oxidative and anti-inflammatory properties. In air polluted environments, a healthy diet with adequate intake of different nutrients may be critical to prevent the development of chronic diseases, particularly those affecting the cardiovascular and pulmonary systems. In addition, an increased intake of antioxidative and anti-inflammatory nutrients may reduce the burden of air-pollution induced oxidative stress and inflammation in persons with chronic inflammatory diseases, thereby providing a possible adjunct to current disease management strategies. Considering the magnitude of the air pollution problem and its health impacts, the potential public health benefits of establishing effective dietary approaches are enormous.

Update of the Multiple Sclerosis Knowledge

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Multiple sclerosis (MS) is a chronic autoimmune disease inducing inflammation, demyelination and axonal damage. It is the most common demyelinating disease of the central nervous system and the second cause of disability in young adults in the western countries.

Although the cause of the disease remains unknown, MS associates with genetic and environmental factors and with inappropriate regulatory responses.

The risk of suffering MS shows a weak association with multiple genes codifying proteins involved in the immune response. It has also been related with high titers of antibodies against Epstein Barr virus or suffering an infectious mononucleosis.

Recently, new data on environmental factors related to MS have arisen. Thus, low levels of vitamin D or melatonin and tobacco smoking associate with higher MS risk and a more severe disease. Obesity at puberty has also been associated with higher probability of MS onset. This could associate with the changes in gut microbiota observed in MS. By contrast, olive oil intake seems to be protective. Finally, age can modify disease course. All these items influence the immune response taking place in MS and can influence patient prognosis.

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Authorship: Luisa M Villar, Immunology Department. Hospital Universitario Ramón y Cajal, Madrid, Spain.

Keywords: Multiple sclerosis, autoimmune diseases, demyelinating diseases.

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What Do We Know About Microbiota? An Update

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Experimental studies using axenic models demonstrated key roles of gut microbes in nutrition, immune function, and body growth of the animal host. Microbial colonizers have widespread influence on host physiology beyond the gastrointestinal tract, including maturation and function of the central nervous system.

The impact of microbial colonization on human health is now being extensively investigated by large-scale research projects aiming at deciphering composition, structure and functions of the microbial communities in human niches, such as gut, mouth, vagina, skin, etc. The field progresses rapidly owing to the availability of high-throughput molecular sequencing techniques combined with powerful bioinformatics for taxonomic identification and comparative analysis of datasets. Loss of species diversity and gene richness in the gut microbiome is commonly reported in individuals affected by chronic non-communicable diseases of increasing incidence in modern society, including metabolic, inflammatory and neoplastic disorders. Whether such microbial changes are cause or consequence of the underlying disorders is still unresolved. Bi-directional influences between host and microbiota have been proven, and some dysbiotic changes are likely to play a role in the origin of disease. While there is epidemiological evidence to support this concept, cohort studies and targeted intervention trials aiming at restoring the ecosystem (microbiota transplantation, antibiotics, diet) are needed for a better understanding of the role of gut microbes in clinical conditions.

Developing and maintaining gut microbiota diversity is a novel clinical target for health promotion and disease prevention. Functional foods and microbiota transplantation are the principal tools for improving host-microbial symbiosis.

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In Vitro Modification of the Peripheral Immune Response of Adult Obese People by Red Grape Polyphenols

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Obesity is a generalized inflammatory status which involves many body's organs, even including cardiovascular and central nervous systems. Our recent data have contributed to a better understanding of the connection between diet, obesity, inflammation. Normal weight children, who had received dietary recommendation to practice a Mediterranean diet (MD), were followed up for one year. At the end of the trial, children were divided into two groups, those who followed MD and those who prevalently ate junk food. The former group exhibited increased salivary levels of interleukin (IL)-10, an anti-inflammatory cytokine, and decreased levels of IL-17, an inflammatory cytokine. Conversely, in the latter group there was a prevalence of IL-17 over IL-10 with an increase of body mass index, thus suggesting a potential tendency to frank obesity in adult life. Polyphenols represent major constituents of MD and are endowed with anti-oxidant and anti-inflammatory activities. On these grounds, in a group of obese adult patients we tested the in vitro effects of red grape polyphenols on their peripheral lymphomonocytes. Patients in comparison to healthy controls exhibited increased absolute numbers of T and B lymphocytes as an indication of a continuous antigenic challenge. Functionally, in these patients the finding of increased levels of IL-21, a cytokine which activates the inflammatory Th17 cell subset, does suggest a polarization of the immune response towards the inflammatory pathway. Polyphenol treatment of obese lymphomonocytes decreased IL-21 levels and release of pro-inflammatory cytokines. Taken together, our data suggest a potential clinical application of polyphenols in the treatment of obesity.

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Keywords: Immunity; Obesity, Polyphenols.

Exercise and Human Brown Adipose Tissue

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The energy burning capacity of brown adipose tissue (BAT) makes it an attractive target for anti-obesity therapies. Moreover, due to its ability to oxidize glucose and lipids, BAT activation has been considered a potential therapy to combat type 2 diabetes and atherogenesis [1]. Recent findings have also shown its potential role on bone metabolism and on inflammation. BAT is mainly regulated by the sympathetic nervous system (SNS), yet recent findings have shown a group of novel activators that act independently of the stimulation of the SNS such as cardiac natriuretic peptides, interleukin-6, β -aminoisobutyric acid and fibroblast growth factor 21 that could influence BAT metabolism. Several strategies are being examined to activate and recruit BAT with no side effects. We postulate that exercise might play a key role on human BAT metabolism through activation of SNS, heart and skeletal muscle [1, 2]. Exercise may also be responsible of the transformation of white adipocyte cells into brown cells, the so called browning effect, yet evidence is mainly available from animal studies. Well-designed and powered intervention studies in humans are needed to elucidate the potential role of exercise in human BAT regulation [2].

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Keywords: Brown adipose tissue; Energy metabolism; Obesity; Thermogenesis; Type 2 diabetes; Body temperature regulation.

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Food, Nutrition and the Immune System: Potential Impact of Global Environmental Change

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The consequences of poor nutrition for foetal, infant and maternal health, growth retardation, reduced immunity, infectious and non-communicable diseases are well known and interrelated. Global demand for food is increasing more steeply than at any other time in human evolutionary history. It is estimated between 9 and 10 billion people will be living on Earth by 2050 who will need sufficient food and nutrition. Global warming, climate change, overfishing, pollution, habitat degradation, loss of biodiversity and urbanisation are important threats to food and nutrition security. Arguably the most important of these human ecological impacts is the health of Earth's Oceans. Marine species are reacting to climate change, overexploitation and pollution faster than terrestrial species e.g. estimates of up to 30–90 per cent of global seafood producing species could be depleted by 2050 [1–3]. Fish and seafood are major sources of animal protein, omega-3 fatty acids, micronutrients e.g. vitamin A, zinc, iron, selenium, iodine etc for an estimated 845 million to 1.4 billion people worldwide who could therefore potentially become vulnerable to nutrient insufficiency i.e. fishing communities in coastal areas and often in developing countries [3]. Populations not primary dependent on seafoods i.e. many developed countries, could also be vulnerable as fish and seafoods have well known brain developmental and functional health benefits and beneficial effects on immune, inflammatory and metabolic systems protecting against e.g. inflammatory and cardiovascular diseases. Long-term health of Oceans depends on international action to reduce global carbon emissions and appropriate management of both wildlife and seafood stocks.

Keywords: nutrition, climate change, overfishing, pollution, biodiversity.

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**N-6 Fatty Acids in Immunity and Inflammation:
Time for a Paradigm Shift in Biology and Medicine**

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Since the 1960's experimental rodent and non-human primate studies have demonstrated reduced atherosclerotic lesions and cardiovascular benefit with the n-6 PUFA linoleic acid (LA, 18:2 n-6) and other studies e.g. suggesting cholesterol lowering effects of LA, lead to recommendations to increase vegetable oil LA intake during the 1970s and early 1980s. More recent meta-analysis, AHA and EPIC-Norfolk findings show n-6 PUFA reduce risk of Coronary Heart Disease (CHD). In contrast, the relatively small Sydney Diet Heart Study revaluation found LA increased rates of CHD, whilst other studies show no associations either positively or negatively. Extensive experimental studies in chronic inflammation and autoimmune mediated inflammation demonstrate protective effects of the n-6 PUFA gamma-linolenic acid (GLA, 18:3 n-6). Reanalysis of clinical trials of LA in Multiple Sclerosis (MS) revealed reduced relapse severity and in mildly affected patients reduced disease progression and three small clinical trials using GLA alone or in combination with other fatty acids report beneficial clinical effects. Furthermore, three clinical trials in Rheumatoid Arthritis (RA) with GLA also show clinically beneficial effects. Moreover, the n-6 PUFA arachidonic acid (AA, 20:4 n-6) is inversely associated with disease activity in MS and RA. Longer chain n-6 fatty acid precursors for eicosanoids can mediate resolution and anti-inflammatory biology e.g. PGJ2, LXA4, PGE1, PGE2, PGI2 and down regulate pro-inflammatory TNF α , IL-1 β , IL-6, IL-8, MCP-1 and ICAM-1 expression and upregulate anti-inflammatory TGF β . The view that n-6 fatty acids are pro-inflammatory is paradoxical and based primarily on the classic role of eicosanoids in early (onset) inflammation [1].

Conflict of Interest: The author is a named inventor on patents for the treatment of neurodegenerative conditions.

Keywords: n-6 fatty acids, immune and inflammatory biology, experimental models, clinical trials.

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**Our Diet, Our Health: Global Opportunities to
Reform the Way We Eat**

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Our diets are changing. The food system from which foods are produced, processed, distributed and ultimately made available for consumption is not delivering the right kinds of foods to promote optimal nutrition and health. Several factors influence changes in the food systems, among these are urbanization, globalization, rapid population growth and government policies. Changes in the food system have led to reduced prices for staple crops and easy accessibility to energy-dense foods high in fat, sugar and salt. Currently about two billion people have micronutrient deficiency, at the same time about 1.9 million people are overweight or obese. Globally non-communicable diseases (NCD) have become the leading cause of death. Of the 56.4 million global deaths in 2015, 70% were due to NCDs. The prevalence of diet-related NCDs is also on the rise in developing countries. Unhealthy diet is an underlying factor for NCDs. There are global opportunities to reform the food systems to deliver on healthy diets. The 2nd International Conference on Nutrition jointly organized by FAO and WHO in November 2014 got Member States to commit to reform their food systems. The 2030 Agenda of the Sustainable Development Goals, especially Goal 2, commits countries to “end hunger, achieve food security and improved nutrition, and promote sustainable agriculture”. The United Nations General Assembly declared the period 2016–2025 as the Decade of Action on Nutrition. These are opportunities to act in repositioning the food systems on the path to deliver the healthy diets needed for optimal nutrition.

Keywords: Food systems, diets, nutrition, SDG.

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**Nutrigenomics and Nutrigenetics Approach to Food
Functionality – A Japanese Perspective**

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Washoku, traditional Japanese cuisine, was registered as a UNESCO's intangible cultural heritage in 2013. As such, the “exceptionally well-balanced and healthy” feature of washoku has been globally renowned. The research on the foods with health-promoting effects (functional foods) gained momentum in early 1980's in Japan. In this country, the world's first policy of legally permitting the commercialization of some specific functional foods was established. Such products are called food for specified health use (FOSHU). In 2015, the Consumer Affairs Agency launched a less strict system that allows producers to make function claims on food labels, Foods with Functional Claims. In either case, scientific evidence for the functionality is essential.

Japanese consume large amount of soy and soy products. We found that genistein, a soy isoflavone, suppressed the expression of

muscle atrophy-related genes in diabetic mice. We then examined whether genistein intake prevents muscle atrophy in a denervation-induced muscle atrophy model. Genistein intake partially suppressed atrophy of soleus muscle and gene expression of Atrogin1 and Murf 1, two ubiquitin ligases involved in muscle atrophy, induced by denervation. DNA microarray analysis identified FOXO1 and the estrogen receptor (ER) as candidates of upstream factors involved in the effect of genistein. Experiments using an antagonist and agonists have revealed that genistein acts through ER α .

In today's talk, some negative aspects relating to current Japanese dietary intake will also be discussed, which include an increase of lean young women as well as increase of low birth weight children. These could be a huge burden to the society in near future.

Keywords: Japan, Functional food, nutrigenomics, soy.

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Omic Sciences and Promotion of Health in the XXI Century

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Molecular nutrition is a relatively new discipline that focuses on the role and interaction of nutrients, foods and bioactive compounds of foods (BACF), with cell receptors, transporters and cell signalling cascades, as well as their interaction with the human genome. Particularly, nutritional genomics is a new science that studies the molecular mechanisms by which nutrients, foods and BACF affect the health of individuals through their interactions with the human genome. Novel "omic" sciences, including nutrigenetics, nutrigenomics, epigenetics, proteomics, metabolomics and metagenomics are key tools to investigate the molecular actions of the diet in humans.

Today, data obtained for the human genome allows us to identify single nucleotide polymorphisms (SNP; copy number variants (CNV) and other genetic variants which are associated to the risks of developing non-communicable chronic diseases and how each subject respond in a different way to the intervention with specific diets enriched in particular nutrients (nutrigenetics). In addition, the action of nutrients and BACF as well as their metabolites can have a different effect on the expression of some genes according to the particular genetic sequence and genome variations (nutrigenomics). Moreover, foods and their components may have a role in the methylation of some DNA bases and affect the methylation pattern and the acetylation profile of histones, as well as in the expression and action of a number of non-coding RNA, e.g micro RNA (miRNA), which in turn can modulate the expression of several genes (epigenetics). Those new sciences and their tools will permit the individual dietary treatment through that we know as "Precision nutrition".

Keywords: Epigenetics, Molecular nutrition, Nutrigenetics, Nutrigenomics, Omic sciences.

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Immunosenescence and Vitamin E Intervention

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Aging process significantly impacts immune system, often in an adverse manner, resulting in a state called immunosenescence, which is believed to be a key contributing factor to the observed higher rates of morbidity and mortality associated with infection in the elderly. In addition to intrinsic changes to the immune system developed with aging, the elderly are more likely to have unmet nutritional requirement due to lower intake and/or increased need of particular nutrients, and this may further impair the aged immune system. Nutritional intervention represents a practical and cost-effective strategy to delay/reverse immune deficit in the elderly, and vitamin E stands out to be one of the most effective micronutrients in this regard. Both animal studies and human trials have shown the immuno-enhancing effect of vitamin E, in particular in T cell-mediated immune response. The clinical relevance of this property of vitamin E is supported not only by its protective effect as evidenced in several experimental infection animal models, but also by the interventional trials in which the elderly receiving vitamin E supplementation have reduced risk of acquiring upper respiratory infections. Furthermore, mechanistic studies have provided insightful evidence to help understand the underlying mechanisms by linking the vitamin E-induced desirable changes in cellular and molecular events in immune cells to the beneficial effect of vitamin E in improving immune function and resistance to infection.

Financial Support: Supported by the USDA under contract #58-1950-4-003.

Keywords: Aging, immunity, infection, vitamin E.

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Lifestyle Consequences on the Immune System in Adolescence

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Adolescence is a decisive period in human life that is linked to key changes in lifestyle habits and psychological functioning determining nutritional requirements, as well as eating behaviour and physical activity patterns. Inadequate lifestyle habits have been shown to promote inflammatory processes. Both eating disorders and obesity are related to medical complications in early stages of life, leading to the development of diseases and alterations of the metabolism and the immune system. The characteristic behaviour patterns that show up during adolescence may produce energy unbalance and nutritional status disturbances. Physical activity, sleep time, and body composition are critical factors for adolescent's health-related behaviours. Prospective controlled trials to assess the protective potential of promoting regular and frequent meals

in children and their families are highly desirable to strengthen the evidence base for such preventive approaches, which should explore the feasibility and effects of interventions. Data from cross-sectional studies have identified several dietary patterns associated with early obesity development, such as meal frequency and distribution, skipping meals, soft drink and fast food consumption, as well as high eating speed. Regular physical activity also seems to offer protection against a wide variety of chronic disease-related risk factors during childhood and adolescence, since it has been shown to exert an anti-inflammatory effect. This is the reason why the combination of adequate physical activity together with healthy dietary habits could be helpful in the prevention of the most common nutrition-related alterations, such as eating disorders and obesity, both with potential distortions in bone mineralization and other clinical and metabolic features. There is growing evidence indicating that particularly moderate-to-vigorous physical activity (MVPA), rather than lower-intensity levels, seems to be independently associated with insulin resistance, blood lipids, blood pressure, inflammatory proteins, and cardiorespiratory fitness in children and adolescents, in a dose-response way. Moreover, the promotion of physical activity together with the reduction of excessive weight during adolescence may reduce exposure to metabolic risk

factors in adolescents. Current recommendations for improving cardiovascular and metabolic health biomarkers in adolescents comprise at least 60 min/day of MVPA. On the other hand, sedentary activities are well known to be associated with increased risk of all-cause and cardiovascular disease mortality. Excessive sedentary time (2/3 of awake hours) has been associated with low cardiorespiratory fitness only in girls and this adverse effect has been shown to be abolished in those girls who meet the recommendations (60 min/day of MVPA). Sleep patterns are also closely linked to several health determinants. Indeed, in apparently healthy adolescents an inverse association has been found between sleep duration and C-reactive protein values, which is an inflammatory marker involved in atherosclerosis, as well as the fact that adolescents who sleep less than 8 h/day show higher adiposity markers, particularly female adolescents. In fact, the most beneficial sleep time range for adolescents is between 8 and 9 hours, which has been related to an anti-inflammatory pattern. Therefore, the study of these lifestyle determinants are essential to implement prevention strategies from the public health perspective.

Keywords: Lifestyle, immune system, adolescence.

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Immunomodulatory Effect of Hesperidin in Immunized Rats*Mariona Camps Bossacoma¹, Àngels Franch²,
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Hesperidin is a flavonoid found in fruits of the genus *Citrus* [1]. It is reported its role in the decrease of Th2-cytokines in murine asthma models [2, 3]. However, the influence of hesperidin on lymphoid tissues and on specific antibody synthesis has not been studied in depth. The aim of this study was to establish the influence of hesperidin on immunized rats. For this, Lewis rats were either orally sensitized with ovalbumin (OVA) and cholera toxin (CT) and fed 0.5% hesperidin diet, or intraperitoneally (ip) immunized with OVA, Bordetella pertussis toxin and Alum and administered by oral gavage 100 or 200 mg of hesperidin per kg of body weight.

In the first experimental design, hesperidin diet modified the effector lymphoid tissue compartments increasing the proportion of TCR $\gamma\delta$ cells among intraepithelial lymphocytes (IEL) and decreasing that one from lamina propria lymphocytes (LPL). Hesperidin diet did not produced modifications on the lymphocyte composition of the inductive sites. In the second experimental design, hesperidin increased the proportion of TCR $\alpha\beta$ ⁺ cells and decreased those of CD45RA⁺ and CD8+CD25⁺ lymphocytes from mesenteric lymph nodes lymphocytes (MLNL). Moreover, hesperidin induced higher production of IFN- γ in stimulated MLNL. Nevertheless, in both designs, the hesperidin did not modify serum specific antibody production neither intestinal IgA content.

In conclusion, hesperidin possesses immunoregulatory properties in the intestinal immune response although the synthesis of systemic antibodies is not affected.

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Authorship: M.C., A.F. and F.J.P.C. designed the study; M.C-B. carried out the experiments, analyzed the data and wrote the abstract; M.C. reviewed the abstract.

Keywords: antibody; flavonoids; hesperidin; immunoregulatory; polyphenol.

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Probiotic Properties and Stress Response of Lactic Acid Bacteria Isolated from Cooked Meat Products*Annel Magdalena Hernández-Alcántara¹, Mari Luz Mohedano¹,
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The aim of this work was to evaluate, for further application in functional food development, some probiotic properties and the response to acidic and heat shock stresses of six lactic acid bacteria (LAB) isolated from Mexican cooked meat products, namely, one *Enterococcus faecium* UAM1 strain and five *Pediococcus pentosaceus* strains.

This study, revealed that under simulated human gastric conditions, the *Enterococcus* strain had a high resistance pattern, superior to that of the *Pediococcus* strains. Exposure to a simulated intestinal stress environment did not greatly affect the survival of any of the LAB strains investigated. In addition, high levels of co-aggregation of the potentially probiotic LAB with Gram-positive and Gram-negative bacterial pathogens were detected. The adherence of *E. faecium* UAM1 to human Caco-2 cell line (around 20%) was significantly higher to that obtained with the five *P. pentosaceus* strains (2%–4%) and *Lactobacillus acidophilus* LA-5 (6%), a commercial probiotic strain.

Thermotolerance, above 65°C, of all of the tested strains was corroborated by DT values. Additionally, a differential proteomic stress response of enterococcal and pediococcal strain was detected by LC-M, upon exposure to heat and acidic stresses. The overall results indicate that *E. faecium* UAM1 has thermo-tolerant properties for survival during the preparation of mexican cooked meat. Moreover, it has probiotic properties that predict its capability to colonize in competition with pathogen the intestinal tract. Thus, this bacterium deserves further investigation for its potential as a component of functional food.

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Authorship: A.M. Hernández-Alcántara performed most of the experimental work and wrote the paper. M.L. Mohedano contributed to the design and performance of the proteomic study. P. López contributed to the design and analysis of the data from the interactomic studies. M.L. Pérez-Chabela contributed to the general design and interpretation of the paper.

Keywords: Lactic acid bacteria, probiotics, interactomic studies, *Pediococcus pentosaceus*, *Enterococcus faecium*.

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Effect of Chronic Sweetener Consumption on BMI, Glucose and Adipokines in Peripheral Blood

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The objective of the present study was to evaluate the effect of chronic sweetener consumption on BMI, glucose and adipokines in peripheral blood. The ingestion of non-caloric sweeteners increases exponentially, since in many commercial products they use as a strategy to reduce calorie consumption [1], control body weight [2] and sometimes glycemia; however its use today is still controversial, as well as the effects of its long-term use [3]. Thirty-two CD1 male mice 21-day old were divided into 4 groups: Control (CL), Sucrose (Suc), Sucralose (SucL) and Stevia (St). BMI, Glucose (mg/dl), TNF- α (pg/mL) and Resistin (pg/mL) were determined. The sweetener was given daily diluted in ultrapure water with the following concentration: Sucrose (41.6 mg/ml), Sucralose and Stevia (4.1 mg/ml), 8–13 h for 84 days. Food and water without sweetener was given ad libitum. BMI and glycemia were recorded weekly, serum TNF- α and Resistin levels were obtained. The BMI ($F = 1,297$, $p = 0.293$) and glucose ($F = 0.923$, $p = 0.443$) remained unchanged at the end of the 84 days of treatment. There was no statistically significant difference in blood levels of TNF- α ($F = 0.571$, $p = 0.639$) and resistin ($F = 1,918$, $p = 0.150$) in any of the study groups. In this study the administration of the sweetener was intermittent (alternating periods with/without exposure), which may explain that there are no significant changes in adipokine blood levels.

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Authorship: Project planning was done by BEMC, the laboratory work was carried out by BEMC, RGC, EHJA, GPLE and abstract writing, reviewing and approval was done by BEMC, GRA, RVR.

Keywords: Non-caloric sweeteners, Body Mass Index, Adipokines.

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Effect of Microalgae Fatty Acids Consumption on the Interferon Gamma Concentrations in Diabetic Mice

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Aim: The aim of the study was to analyze the effect of Omega-3 fatty acids extracted from microalgae on the IFN- γ concentrations. Diabetes Mellitus is considered a chronic disease characterized for an inflammatory state in which many cytokines are continuously released including Interferon gamma (IFN- γ) [1]. Omega-3 fatty acids are well known for reducing inflammatory states such as in diabetes and contribute to stop the development of complications.

Method: 30 db/db male mice 8 weeks old were randomly assigned either to a control group (CL); a supplemented with Lyophilized microalgae EPA+DHA fatty group (LY); a supplemented with saturated fatty acids (pure coconut oil) group (SAT) or a group fed with a microalgae EPA+DHA modified diet (MD). Experimental study was conducted from the 8th to the 16th week of life. After treatment, blood lymphocytes were isolated and flow cytometry was conducted.

Results: One-way ANOVA was performed and showed significant differences between treatments ($F = 1009.208$, $p < 0.001$). LY and MD group showed significantly lower concentrations when compared to Control group (Bonferroni, $p < 0.001$) and SAT group was significantly higher also when compared to Control group (Bonferroni, $p < 0.001$).

Conclusions: The consumption of microalgae Omega-3 fatty acids whether from diet or supplemented decreases IFN- γ concentrations which may reduce the risk of complications in Diabetes Mellitus.

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Authorship: Project planning was done by BEMC, the laboratory work was carried out by BEMC, GPLE, EHJA, MVT and abstract writing, reviewing and approval was done by BEMC, GRA, RVR.

Keywords: Microalgae, Fatty acids consumption, Interferon gamma.

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Cocoa Polyphenols Enhance “In Vitro” the Interferon Immune Response Pathways

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Cocoa consumption is associated with beneficial effects on human health. This has been attributed to its polyphenol components, which have been shown to decrease the risk of cardiovascular and inflammatory diseases, metabolic disorders, and to play a role in cancer prevention [1]. We had previously demonstrated by in vitro and in vivo assays that cocoa also influences immune response [2]. In this line, our working hypothesis was that cocoa components could be involved in the cytokine interaction of epithelial and immune cells located in the intestine. To test this idea, we evaluated the effect of a polyphenol cocoa extract on gene expression, by using Affymetrix microarrays, of an intestinal cell line (the colonic HT-29 cells). We observed that a total of 134 genes were up-regulated and 17 genes were down-regulated in HT-29 cells upon incubation with cocoa extract. Among the genes differentially expressed, 48 were identified as interferon regulated genes using the INTERFEROME database. Furthermore, a biological associated network (BAN) constructed using the Pathway Architect software revealed that STAT1, IF44 and AGT were all highly interconnected nodes. Additionally, the changes in the expression of

several Interferon regulated genes were also determined using the PCR array technology. In THP-1 cells, a human monocyte derived cell line, cocoa extract increased the levels of IRF-1, IRF-8 and STAT1, three interferon inducible genes. Overall, our results indicate that the immunomodulatory effect of cocoa is partially mediated through its broad induction of interferon related genes.

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Authorship: The authors’ contributions were as follows: C.O, C.J.C and V.N conceived and designed the research. C.O. performed the experiments and all the authors were involved in the analysis and interpretation of the data.

Keywords: cocoa, microarray, interferon, STAT1, immunity.

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Discerning the Role of Nutritional Protease Inhibitors in Hepatoblastoma Cells and Macrophages’ Phenotype

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Proteinase inhibitors, present in several cereal grains and legumes, have been revealed as innate immune modulatory factors (imPIs) through their interaction with the toll-like receptor (TLR)-4. TLR4 is required for hepatocarcinogenesis in the chronically injured liver. Notably, non-bone marrow-derived intrahepatic macrophages mediate the tumor-promoting effect of TLR4. This study aims to monitor TLR4-mediated innate immune responses for several imPIs from *Triticum aestivum* (TA), *Avena sativa* (AS) and ‘whole’ (bran) *Oryza sativa* (OS). Hepatoblastoma (HB-8965[®]) and humanized macrophage-like (HB-8902[®]) cells were used to perform proliferation assay, cytokine (IL-6, IL-10 and TNFα) production, endosomal acidification and quantify oxygen

consumption rates (OCR) in a mitostress assay. All imPIs shared TLR4-inducing expression capability in both HB-8965[®] and HB-8902[®] cells with highest values in cultures exposed to OS. In HB-8902[®] cells, TLR4 expression was associated to significantly different increased expression values for cathepsin-E and lipooxygenase-2. After imPIs challenge, only HB-8902[®] cells showed significant increases in OCR associated to non-mitochondrial respiration. These metabolic changes were associated to increased endosomal acidification due to impairment of lysosomes maturation that could promote different dimerization modes for TLR4. ImPIs favored different cytokines production patterns being quantified the lowest values in cultures exposed to AS. Differential imPIs-induced TLR4 expression in HB-8965[®] cells mediate innate immune responses that influence tumor cell growth. In HB-8902[®] cells there was evidenced an imPIs-promoted metabolic switch required for a pro-tumoral macrophage phenotype development. The latter can be associated to changes in important regulatory factors of the CD4+ T cells priming and effector CD8+ T cells.

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Authorship: LL: acquisition of data; analysis and interpretation of data. MGdC: acquisition of data and analysis. ARdM: acquisition of data and analysis. JML: study design; acquisition of data; analysis and interpretation of data.

Keywords: Proteinase inhibitors, toll-like receptor-4, hepatoblastoma, macrophages, cereals.

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Immunomodulatory and Anti-Inflammatory Activities of a Wheat Gluten Protein Hydrolysate

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The aim of this work was to evaluate the potential immunomodulatory and anti-inflammatory activities of a wheat gluten protein hydrolysate (WPH) obtained by enzymatic hydrolysis of wheat gluten with Alcalase, at pH 8 and 50°C for 60 min. Recently, it has been suggested that angiotensin converting enzyme (ACE) plays a role in the immune response, and ACE inhibitors have been reported to attenuate immune disorders [1]. Inhibition of ACE was measured as described by Sentandreu & Toldrá [2], finding that WPH inhibited more than 60% of ACE activity.

Anti-inflammatory activity was tested in a THP-1-derived macrophage model by measuring cytokines' expression by quantitative PCR. Before testing anti-inflammatory properties, THP-1-derived macrophages were exposed to RPMI medium containing WPH at several concentrations and cell viability and the potential cytotoxicity of the WPH was evaluated. The results showed that WPH do not compromise the integrity of THP-1-derived macrophages. On the other hand, WPH induced the downregulation of the pro-inflammatory cytokines TNF, IL-1 β and IL-6 in activated macrophages by 55, 15 and 23%, respectively.

In conclusion, WPH could be used in the formulation of functional foods with immunomodulatory activity.

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Authorship: F Millán and J Pedroche formulated the research questions and designed the study. MC Millán and MM Yust carried it out and analysed the data. MM Yust wrote the abstract.

Keywords: Wheat gluten; enzymatic hydrolysis; bioactive peptides; anti-inflammatory activity.

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Effect of Adipokines Supplementation on the Intestinal Lymphocyte Composition in Suckling Rats

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It is in early life when the development of the immune system undergoes the most important changes [1]. Leptin and adiponectin are metabolic hormones or adipokines found in breast milk [2]. The immune activity of these adipokines has been described in *in vitro* studies [3], but few evidences are reported in *in vivo* approaches. The aim of this study was to establish the influence of a nutritional supplementation with leptin or adiponectin on the intestinal immune system, particularly in the lymphocyte composition of mesenteric lymph nodes (MLN) and intestinal intraepithelial lymphocytes (IEL). For this purpose, suckling Wistar rats were daily supplemented with leptin, adiponectin or vehicle during suckling period (21 days). MLN cell and IEL were isolated to establish their lymphocyte composition by immunofluorescence staining and flow cytometry analysis. At day 14, leptin supplementation significantly increased the TCRαβ⁺ cell proportion in MLN. Moreover, on that day, both leptin and adiponectin increased the percentage of CD8⁺ cells in MLN. Regarding IEL population, at the end of suckling period, both adipokines increased the proportions of TCRαβ⁺, CD8⁺ and NKT cells. These changes were associated with an increase in a more intestinal phenotype (CD8αα⁺) induced by leptin supplementation or a more peripheral phenotype (CD8αβ⁺) produced by adiponectin administration. Overall, these results suggest that leptin and adiponectin promote the maturation of the intestinal immune system during the suckling period.

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Authorship: A.F., F.J.P.-C., M.C. and M.J.R.-L. conceived and designed the experiments; B.G.-P. and M.A.-G. carried out the experiments, analysed the data and wrote the abstract; A.F., F.J.P.-C., M.C. and M.J.R.-L. reviewed it.

Keywords: leptin, adiponectin, suckling rat, intraepithelial lymphocytes, mesenteric lymph nodes.

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Leptin and Adiponectin Supplementation to Suckling Rats Modulates the Functionality of Lymph Nodes Lymphocytes

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Human breast milk contains a wide variety of bioactive factors such as leptin and adiponectin which are important for newborn development [1]. It is well described that they are involved in the control of endocrine and metabolic systems, nevertheless, in addition to their metabolic activities, they could also contribute to the regulation of innate and adaptive immune responses [2]. The aim of the present study was to establish the effect of leptin and adiponectin supplementation to suckling rats on the lymphocyte capacity of proliferation and cytokine secretion *ex vivo*. For this purpose, Wistar rats were daily supplemented by oral gavage with leptin, adiponectin or vehicle since the day of birth until the end of the suckling period (21 days). Lymphocytes from mesenteric lymph nodes collected that day were isolated in order to be stimulated with anti-CD3/CD28 antibodies and cytokine production was quantified in culture supernatants. Adiponectin supplementation raised lymphoproliferative ability, while leptin did not modify it. Regarding cytokine secretion, both adipokine supplementations decreased IL-2 and IL-12p70 in comparison with reference group. In addition, IL-13 production from animals who received the adiponectin administration also decreased. In summary, these results demonstrate that the supplementation with breast milk adipokines in early life modifies lymphocyte proliferation and cytokine secretion, suggesting a modulatory role on the immune system development.

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Authorship: A.F., F.J.P.-C. and M.C. conceived and designed the experiments; B.G.-P., M.A.-G. and L.M.-M. carried out the experiments; B.G.-P., M.A.-G. analysed the data and wrote the abstract; A.F., F.J.P.-C. and M.C. reviewed it.

Keywords: Leptin, adiponectin, breast milk, lymphocyte proliferation, cytokines.

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Growth Factors Supplementation Influences the Intestinal Lymphocyte Functionality in Suckling Rats

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The newborn immune response is functionally deficient and less competent compared with adults [1]. Breast milk provides a large number of compounds that contribute to the maturation of the immune system in early life such as specific growth factors [2]. The aim of the present study was to ascertain the influence of transforming growth factor (TGF)- β 2, epidermal growth factor (EGF) and fibroblast growth factor 21 (FGF21), which are present in breast milk, on the development of the intestinal immune system during suckling. For this, newborn Wistar rats were randomly distributed into four experimental groups: Reference, TGF- β 2, EGF and FGF21. Rats were daily supplemented by oral gavage with these growth factors since the day of birth until the end of the suckling period (day 21). At days 14 and 21 of life, lymphocytes from mesenteric lymph nodes were isolated, cultured and their proliferative ability as well as their cytokine secretion were evaluated. The results showed that only FGF21 supplementation on day 21 was able to promote the lymphoproliferation. Regarding cytokine production, it was on day 21 when changes were more patent. EGF and FGF21 decreased IL-13 levels whereas both TGF- β 2 and EGF reduced IL-10 and IL-4 production. Overall, EGF decreased the IL-10/TNF α ratio and increased the Th1/Th2 (IFN γ /IL-4) ratio when compared to the reference group. These results evidence that TGF- β 2, FGF21 and mainly EGF supplementation have an immunoregulatory effect in early life promoting the switch from Th2 to Th1 responses and then contributing to the maturation of neonatal intestinal immune system.

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Authorship: A.F., F.J.P-C. and M.C. designed the study; P.T-C., B.G-P., and M.A-G. carried out the experiments; P.T-C. and

B.G-P., analyzed the data and wrote the abstract; A.F., F.J.P-C. and M.C. reviewed it.

Keywords: Growth factors, suckling rat, lymphoproliferation, mesenteric lymph nodes, cytokines.

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Probiotic Properties of EPS-Producing *Pediococcus* Strains

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Pediococcus parvulus 2.6R [1] and *Pediococcus ethanolidurans* ZEp are two exopolysaccharide-producing lactic acid bacteria (LAB) isolated from Basque country cider. Both *pediococci* produce a homopolysaccharide (HoPS) characterized as 2-substituted (1,3)- β -D-glucan with prebiotic properties [2]. In addition, *P. ethanolidurans* ZEp also synthesizes a heteropolysaccharide (HePS), which is composed of glucose, galactose, N-acetylglucosamine and phosphoglycerate. Chemical mutagenesis generated the isogenic strains: *P. parvulus* 2.6NR [3] and *P. ethanolidurans* ZEXr with null or reduced 2-substituted (1,3)- β -D-glucan production, respectively.

The aim of this work was to characterize the influence of the exopolysaccharides on the probiotic properties of these LAB as well as the immunomodulatory properties of the 2-substituted (1,3)- β -D-glucan. As expected from previous results [4] for *P. parvulus* strains, synthesis or addition of the HoPS resulted in a significant increase of the bacterial adhesion to human Caco-2 cells, while for *P. ethanolidurans* strains the binding to the enterocytes was higher for ZEXr than for ZEp. The positive influence of the HoPS on *P. parvulus* adhesion to intestinal cells was validated using an in vivo gnotobiotic zebrafish model and fluorescently labelled 2.6R and 2.6NR strains. In addition, in this model 2.6R showed a greater ability than 2.6NR to compete with *Vibrio anguillarum*, protecting zebrafish against infection. Finally, in vitro/vivo studies with either human cell lines or zebrafish, plus determining levels and expression of cytokines indicated an anti-inflammatory effect of the 2-substituted (1,3)- β -D-glucan. Therefore, *P. parvulus* 2.6R and its HoPS seem to have potential to produce functional food designed for people with intestinal disorders.

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Authorship: A. Pérez-Ramos performed the experimental work regarding the *P. parvulus* strains and their HoPS and wrote the paper. M.G. Llamas performed the work related to *P. ethanolidurans* strains and their EPS. M.L. Mohedano contributed to the design of the interactomic studies. M.A. Pardo was responsible for the design of the experiments involving the zebra fish embryos. M.T. Dueñas designed and analysed the experiments related to *P. ethanolidurans* strains and their EPS. P. López contributed to the design and analysis of the experiments performed with *P. parvulus* strains and their HoPS.

Keywords: Lactic acid bacteria, probiotics, polysaccharides, *Pediococci*, β -glucan.

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A Multispecies Probiotic Improves Cognitive Function, Risk of Falls and Inflammatory Response in Cirrhotic Patients

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Aim: To evaluate the effect of the multispecies probiotic mixture De Simone Formulation [DSF] on cognitive function, risk of falls and inflammatory response in patients with cirrhosis.

Patients and Methods: In this double-blind placebo-controlled clinical trial (NCT01686698), we included outpatients with

cirrhosis and cognitive dysfunction (Psychometric Hepatic Encephalopathy Score [PHES] <-4) and/or falls in the previous year. Patients were randomized to receive DSF (Vivomixx®) one sachet containing 450 billion bacteria bid for 12 weeks or placebo. We evaluated the changes in cognitive function (PHES), risk of falls (gait speed, Timed Up & Go-test [TUG] and incidence of falls), systemic inflammatory response (CRP, TNF- α , IL-6, IL-10, neutrophil oxidative burst), bacterial translocation (serum bacterial DNA and lipopolysaccharide binding protein [LBP]), intestinal barrier (fatty acid binding protein [FABP]-6 and 2 and zonulin in serum and urinary claudin-3), and fecal microbiota.

Results: We included 36 cirrhotic outpatients. Patients treated with DSF showed a significant improvement in PHES ($p = 0.01$), gait speed ($p = 0.03$), TUG ($p = 0.02$) and a trend to a lower incidence of falls during follow-up (0% vs. 22.2% in the placebo group, $p = 0.10$). In the probiotic group, we observed a decrease in FABP-6 ($p = 0.009$), claudin-3 ($p = 0.002$), CRP ($p = 0.01$) and TNF- α ($p = 0.01$), and an increase in poststimulation neutrophil oxidative burst ($p = 0.002$). No significant changes in serum bacterial DNA and LBP or fecal microbiota were observed.

Conclusions: The multispecies probiotic DSF improves cognitive function, risk of falls, inflammatory response and intestinal barrier in patients with cirrhosis and cognitive dysfunction and/or previous falls.

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Authorship: Eva Román and Germán Soriano formulated the research questions, designed the study and analysed the data and they wrote the abstract.

Juan Camilo Nieto and Silvia Vidal performed the analysis of the parameters of systemic inflammatory response, intestinal barrier and oxidative burst.

Germán Soriano and Carlos Guarner selected the outpatients in Hospital de Sant Pau.

Eva Román carried out the tests of cognitive function, risk of falls, quality of life and collected all the samples.

Chaysavanh Manichanh analyzed the fecal microbiota at the Vall d'Hebron Research Institute.

Keywords: Probiotics, cognitive function, cirrhotic patient, risk of falls, inflammatory response.

Characterization of Exopolysaccharide-Producing Lactic Acid Bacteria of Technological Interest

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Objective: The characterization of lactic acid bacteria (LAB), belonging to *Leuconostoc mesenteroides* and *Lactobacillus sakei* species isolated from different ecosystems, and of their exopolysaccharides (EPS) produced in the presence of sucrose as carbon source.

Key Findings: LAB isolated from food products (camel and sheep milks, meat products and cider) were selected according to the level of production of EPS in both liquid as solid media. EPS were produced in the presence of sucrose, purified from bacterial culture supernatants by ethanol precipitation, dialyzed and lyophilized [1]. Methylation analysis and determination of monomer composition revealed that the EPS are homopolysaccharides (dextrans) with a main chain of glucopyranose units linked by α -(1,6) bonds, and partially branched in the O-3 position by a single α -glucopyranose unit [2]. The determination of the rheological properties of the dextrans showed that dextrans, under shear conditions, have a pseudoplastic behavior linked to a flexible chain influenced by the polymer concentrations. Size exclusion chromatography coupled with multiangle laser light scattering (SEC-MALLS) detection analysis [3] demonstrated that the dextrans had molecular masses between 1.74×10^8 Da and 4.41×10^8 Da. In addition, these dextrans clearly showed in vitro anti-inflammatory immunomodulatory activity in the THP-1 cell line. The ability of the dextran-producing LAB for binding to Caco-2 cells was strain dependent and in some cases negatively affected by the production of dextran as previously observed [4].

Conclusion: The LAB and their dextrans showed probiotic and technological properties that qualified them to be used in the agri-food for industry producing functional foods.

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Authorship: K. Zarour: Isolation, technological characterization of *Leuconostoc* strains from Algerian milks and their exopolysaccharides. M.G. Llamas: Isolation, technological characterization of *Leuconostoc* and *Lactobacillus* strains from meat products and cider and their exopolysaccharides. R. Aznar provided the strains isolated from meat products and carried out a critical reading of the paper. M.T. Dueñas: contribution in interpretation of

rheological analysis data of polymers produced by LAB. M. Kihal: contribution in isolation and phenotypic identification of *Leuconostoc* strains from Algerian food products. P. López: research direction and interpretation of the analytical data.

Keywords: Lactic acid bacteria, exopolysaccharides, dextrans, adhesion, immunomodulation.

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Seeking a Model for Immunonutrition: Exhausting Exercise in Rats

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Although a regular and moderate physical activity enhances body defences (Kruijsen-Jaarsma et al., 2013), the immune system is negatively impacted by exhausting exercise, conducting an increasing risk of opportunistic infections (Gleeson, 2007). This condition is therefore a challenge for immunonutrition, looking for appropriate diets that prevent, delay or inhibit the deficiencies in the immunity. Animal models in which an exercise-derived immunodepression could be reproduced are very limited. The aim of this study was to establish immune markers of exhausting exercise both in the systemic and mucosal lymphoid tissues of rats reflecting the depression of the immune function.

Young Wistar rats were trained in a treadmill for 4 weeks and, at the end, they were submitted to an exhaustion protocol in the same treadmill. Blood and faeces were collected throughout the study. Macrophages were isolated from peritoneal cavity and small intestine was dissected. In addition, the weight of gastrocnemius muscle, heart and lymphoid tissues were registered.

Results showed that runners had higher gastrocnemius muscle and heart weights than sedentary rats, but the spleen and thymus weight decreased in runner rats. Macrophages from runners produced higher reactive oxygen species. Moreover, faecal IgA content decreased by training. The gene expression in small intestine tissue was altered: occludin mRNA was reduced in runner rats whereas that of mucin increased.

In conclusion, the increasing training exercise applied in rats for 4 weeks followed by an exhausting session produced a harmful effect on systemic and intestinal immune system that can be target for immunonutrition studies.

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Authorship: M.C., F.P.-C. and A.F. designed the study, S.E.A., I.A. and M.A.-G. carried it out, S.E.A. analysed the data, M.C. and S.E.A. wrote the abstract.

Keywords: Exhausting exercise, mucosal immune system, lymphoid tissues.

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Immunonutritional Protease Inhibitors from Rice: A Metaproteomic Analysis

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Non-celiac gluten sensitivity (NCGS), whose diagnosis has sometimes been mistaken with celiac disease [1–4], could be present in 5–10% of the population [5]. The specific action mechanism is still unknown, although some studies point to α -amylase/trypsin inhibitors, mainly found in wheat varieties that contain gluten, as potential responsible for the effect [6]. In addition, this inhibitors have been observed to be strong activators of Toll-like receptors-4 [7], suggesting an alteration of the innate immune response [8]. In the scientific literature, one of the most characterized is the extracellular protein P17314 (entry in UniProtKB/Swiss-Prot, ExPASy database), reported in the organism *Triticum aestivum* (wheat) [9]. To date, the treatment for NCGS disease consist in the prescription of a gluten-free diet for life [3, 10–12], usually recommending other alternative cereals such as rice (*Oryza sativa* subsp. *japonica*) in exchange. However, this cereal contains proteins of the α -amylase/trypsin inhibitors family [13], such as Q8H4L8 and Q8GVI3, among others, which have a high sequence homology

with the protein P17314, 52% and 63% respectively, and could trigger the immune response associated with NCGS. In this metaproteomic analysis, rice proteins that could trigger the immune response associated with NCGS have been studied. Also, peptides released from the studied proteins after simulated digestion with different gastric enzymes have been evaluated (PeptideMass, ExPASy database). This analysis reveals the importance of adequate nutritional recommendations in this kind of patients, since only adherence to a gluten-free diet can not be effective in improving the symptoms associated with NCGS.

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Authorship: Both authors declare to have contributed equally in the formulation of the research questions, design, elaboration, results analysis and writing in this study.

Keywords: Non-celiac gluten sensitivity, α -amylase/trypsin inhibitors, *Oryza sativa*, *Triticum aestivum*, metaproteomic analysis.

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Non-Digestible Polysaccharides Polarize Human Macrophages Towards a Distinct Subtype

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Non-digestible polysaccharides (NDPs) have been attributed with beneficial health effects. Part of these effects might stem from immunomodulation of intestinal residing immune cells. Macrophages represent a key immune cell-type in regulating intestinal homeostasis and have been demonstrated to extent protrusions between epithelial cells to directly sample luminal content and phagocytose NDPs. Homeostasis is partly regulated by interplay between tolerant macrophages, which reside in submucosal regions, and inflammatory macrophages, which accumulate upon an infection. To investigate the immunomodulatory potential of NDPs we used an in vitro macrophage model system mimicking tolerant (M(IL-4)) and inflammatory (M(TNF α +IFN γ)) macrophages. Gene-based analysis revealed that stimulations with NDPs results in the generation of an alternative subtype of macrophage to M(IL-4) and M(TNF α +IFN γ), termed M(NDP). Typical M1 characteristics, such as IDO-mediated kynurenine production, and M2 characteristics, such as enhanced phagocytosis, are down-regulated in M(NDP). In contrast, a specific set of chemokines was found to be upregulated, including CCL1, CCL5, CCL20, CCL24, CXCL8 and IL-1 β . M(NDP) spend-medium containing these chemokines significantly enhanced migration of monocytes when compared to non-polarised-macrophage spend-medium. As the tolerant macrophages constitute the main subtype found in the intestine in homeostasis, we also investigated whether this alternative subtype could be induced when stimulating polarised M(IL-4). Indeed, NDPs prove to re-polarise M(IL-4) towards M(NDP) which spend-medium again demonstrated a significant enhanced migration of monocytes. This data shows that NDPs could mediate immunomodulation by polarising macrophages towards an alternative macrophage subtype.

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Authorship: Y.T., C.G., and J.J.M. conceived and designed the experiments. Y.T., C.G. and E.H.S. performed the experiments. Y.T., C.G., and J.J.M. analysed the data. Y.T., C.G., J.J.M., and H.J.W. wrote the manuscript.

Keywords: Intestinal immunity, macrophage polarisation, non-digestible polysaccharides, gene expression markers, chemotaxis.

Two Bifidobacteria of Human Origin That Have Potential as Components of Functional Food

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The aim of this work was the evaluation of the probiotic and technological properties of two Bifidobacteria isolated from infant faeces, Bifidobacterium longum INIA P132 and Bifidobacterium infantis INIA P731, as well as the characterization of the exopolysaccharides (EPS) produced by them. Both strains showed moderate resistance to gastrointestinal conditions when compared to commercial probiotic bacteria used as control, Bifidobacterium animalis subsp. lactis BB12 and Bifidobacterium longum BB536. Moreover, the P132 strain grew in milk better than the reference strains and had equal tolerance to freezing and freeze-drying. The P731 strain demonstrated a higher adhesion capacity to human Caco-2 cells than the probiotic strain BB12 [1]. The structural characterization [2] of the EPS from the two bifidobacteria revealed in both preparations two fractions with different molecular mass (Mw), in the range of 106 and 104 Da for B. longum and 105 and 104 for B. infantis. The analysis of their monosaccharide composition showed that both bacteria produce heteropolysaccharides (HePS) composed of rhamnose, galactose and glucose. The two EPS preparations were able to immunomodulate human PMA-THP-1 macrophages [3] and determination of the levels of TNF- α and IL-10 secreted by the macrophages after treatment with EPS indicate that they possess anti-inflammatory activity. In addition, in a DSS induced enterocolitis zebra fish model, these HePS were able to reduce larva mortality. Finally, as B. longum P132 and B. infantis P731 showed interesting technological properties, both bifidobacteria and their HePS are potential candidates for the production of functional food.

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Authorship: M.G. Llamas performed the experimental work on the probiotic characterization of Bifidobacteria and their EPS and wrote the paper. A. I. Puertas performed the experimental work on the zebrafish model. M.A. Pardo was responsible for the design of the experiments involving the zebra fish embryos. A Prieto was responsible for the design of the experiments involving physicochemical characterization of the EPS. E. Rodríguez was responsible for the design of the experiments involving the techno-

logical properties of the Bifidobacteria. P. López contributed to the design and analysis of interactomic studies. M.T. Dueñas performed the overall design and analysis of the experiments.

Keywords: Bifidobacteria, probiotics, polysaccharides, functional food.

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Impact of High-Lipid Diet on Serum and Thymus' Fatty Acid Profiles of Growing Rats

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The thymus shows important functional changes in response to nutritional disorders. The aim of this work is to analyze the effect of high-lipid diet from different sources, on serum and thymus' fatty acid profiles of growing rats. Weanling Wistar rats were fed during 40 days 42% dietary fat provided by butter (B group); high oleic sunflower oil (HO group) and sunflower oil (S group). Control group (C) received normocaloric diet (AIN'93). Serum and thymus fatty acids profiles were determined by gas chromatography. Statistical analysis used ANOVA and Dunnett's post test. Results of oleic, linoleic and α -linolenic (ALA) acids expressed as %Area (* $p < 0.01$) were:

Serum: Oleic: B = 20.4 ± 2.2 ; HO = 27.0 ± 3.7 ; S = 11.5 ± 3.9 ; C = 11.3 ± 2.2 ; Linoleic: B = 9.8 ± 1.1 ; HO = 7.4 ± 1.9 ; S = 30.9 ± 2.6 ; C = 18.3 ± 3.3 ; ALA: B = 0.4 ± 0.1 ; HO = 0.3 ± 0.1 ; S = 0.3 ± 0.1 ; C = 0.8 ± 0.2 . Thymus: Oleic: B = 16.1 ± 6.3 ; HO = 36.1 ± 10.7 ; S = 14.9 ± 1.9 ; C = 16.8 ± 5.2 ; Linoleic: B = 3.7 ± 0.6 ; HO = 4.0 ± 0.6 ; S = 29.5 ± 1.2 ; C = 10.2 ± 3.1 ; ALA: B = 0.3 ± 0.1 ; HO = 0.3 ± 0.1 ; S = 0.3 ± 0.1 ; C = 0.7 ± 0.1 .

B and HO groups showed lower α -linolenic and linoleic acids levels in serum and thymus. Oleic acid increased in both groups in serum but only increased in HO thymus. The results suggest that it was exacerbated $\omega 9$ family with diminution of essential fatty acids. S group presents high levels of linoleic acid and lower levels of α -linolenic acid in serum and thymus. The results suggest that the sources of dietary lipids provoked changes in serum and thymus fatty acids profiles.

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Authorship: The authors contributed equally in the design, realization and writing of the work.

Keywords: Thymus, fatty acids, lipid, rat, diet.

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Effects of Lactobacillus Fermentum CECT5716 Supplementation in Pregnant and Breastfeeding Rats

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Little is known about the beneficial effects that probiotics exert in mothers during the pregnancy and breastfeeding periods. Among probiotics of interest, *Lactobacillus fermentum* CECT 5716, a strain isolated from human milk, could be a putative candidate for reinforcing immunological functions as it possesses an immunoregulatory activity and increases immunological responses in healthy adults [1, 2]. Therefore, the aim of the present study was to elucidate whether probiotic supplementation in rats during both periods could influence anthropometric and immunological parameters. For this purpose, Lewis rats were daily administered either with *L. fermentum* CECT5716 (probiotic group) or vehicle (REF group) beginning the day of gestation and during the first two weeks of breastfeeding. At the end of the intervention, lymphocytes from mesenteric lymph nodes (MLN) were phenotyped by immunofluorescence staining and flow cytometry analysis. Proliferation and phagocytosis assays were also performed. The *L. fermentum* CECT5716 supplementation resulted in a lower proportion of TCR $\alpha\beta$ + lymphocytes compared to the REF group ($p < 0.05$) mainly due to a significant decrease in cytotoxic (CD8+) T cells. This reduction was also associated with a decrease in CD8 $\alpha\beta$ + lymphocytes ($p < 0.05$). Moreover, helper (CD4+) T cells presented higher expression of the activation marker CD25+ in lymphocytes from the probiotic group. No effects were observed either on the proliferative or phagocytic activities of the MLN lymphocytes. Furthermore, probiotic supplementation did not modify the anthropometric parameters analyzed. These results demonstrate that *L. fermentum* CECT5716 supplementation

is able to modulate the intestinal immune phenotype in pregnant and breastfeeding rats.

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Authorship: The authors' contributions were as follows: F. J P.-C, M.C, A.F and M. J. R.-L conceived and designed the research. The experiments and the analysis and interpretation of the data was carried out by I. A.-B, M. M.-C, F. J P.-C and M. J. R.-L.

Keywords: Probiotic, immune system, lymphocyte, breastfeeding, phenotype.

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"In Vivo" Screening of Five Probiotics for the Treatment of Rotavirus Diarrhoea

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Rotavirus infection is responsible for causing severe acute gastroenteritis worldwide, being children <5 years old at greatest risk for developing diarrhoea. Recent studies [1–4] suggest the use of probiotics for the amelioration of this clinical process.

The aim of this study was to determine in the rat rotavirus-induced diarrhoea model the specific modulatory effects of five probiotics, being all Bifidobacterium and Lactobacillus strains isolated from different biological sources.

For that, suckling rats were supplemented with the probiotics from the days 3 to 14 of life. All groups received the RV intragastrically at day 5 of life. Clinical parameters were evaluated daily from the direct observation of the faecal consistency, scoring the faeces from 1 to 4, depending on colour, consistency and amount of water present. Other variables such as the weight, viral load and pH of faeces were evaluated.

Two of the probiotics, one Bifidobacterium and one Lactobacillus strains, were the most effective regarding the amelioration of

incidence, severity and duration of diarrhoea. Moreover, all probiotics, and specially one of the above, decreased the faecal output compared to RV. Furthermore, only the Lactobacillus strains were able to reduce the detection of viral particles in faeces the day after inoculation, suggesting a pathogen aggregation function.

These results evidence the strain specific effect of probiotics on the RV associated diarrhoea and the differential mechanisms of action. Thus, this model has allowed us to select the most interesting candidates for future research in the treatment of rotavirus diarrhoea.

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Authorship: The authors' contributions were as follows: F. J P.-C, M.C, A.F and K.N conceived and designed the research. The experiments and the analysis and interpretation of the data was carried out by I. A.-B, M. M.-C and F. J P.-C. All authors collaborated in the abstract writing.

Keywords: Probiotic, immune system, rotavirus, diarrhoea, lactobacillus.

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The Identification and Functional Characterization of Lactobacillus Strains Producing Antibacterial Compounds Used as a Technological Barrier for Food Safety

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Objective: The Identification and technological-functional characterization of autochthonous Lactobacillus strains isolated from algerian raw camel and goat milks, and study of their ability

to inhibit the growth of pathogenic strains by the production of antibacterial compounds.

Key Findings: The identification of isolated lactic acid bacteria strains allowed us to identify five strains of *Lb. plantarum*, and a one strain of *Lb. johnsonii* [1]. From a technological point of view, the strains possess a high proteolytic activity, produce aroma compounds and metabolize citrate, which help to improve the organoleptic properties of fermented food [2]. However, in skimmed milk, *Lb. plantarum* LbG 22 strain acidified the medium at a rate of -0.033 pH units/hr and produced lactic acid at a rate of 0.77 mM/hr. Compared to the other bacteria studied, this strain has the greatest inhibitory activity against food-borne pathogenic strains of *Staphylococcus aureus* ATCC 25923 and *Listeria ivanovii* ATCC 19119, Which pose a potential danger to the consumer's health [3]. The study of the chemical nature of inhibiting agent shows that this strain possesses an important antibacterial activity, which is due, mainly, to a bacteriocin-like compound. In addition to lactic acid production. In skimmed milk, LbG 22 inhibits the growth of *S. aureus* ATCC 43300 after 24 h of mixed culture incubation.

Conclusion: The strain *Lb. plantarum* LBG22 has shown a good technological and functional properties that qualified it to be used as good probiotic candidates for preservation and conservation in the agri-food for industry.

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Authorship: A. Z. Boumehira: Isolation, technological-functional characterization of lactic acid bacteria strains. M. Kihal: research direction and interpretation of the analytical data.

Keywords: Lactic acid bacteria, *Lactobacillus plantarum*, Antibacterial compounds, Probiotics, Milk.

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Proinflammatory Cytokines Determination and Gene Expression of Catalase in People with Obesity After Dietary Intervention

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Aim: To evaluate the peripheral concentration of TNF- α and IL-6 and the gene expression of antioxidant enzyme catalase (CAT) in mononuclear cells in obese participants after a dietary intervention with high content of fiber.

Methods: The study included Mexican participants selected from the Clinic of Nutrition of Health Department of the Universidad Iberoamericana from Mexico City. 21 participants were included in the study with IMC >30 . Clinical, anthropometric and biochemical variables were evaluated in participants and received a dietary intervention with daily consumption of “nopal” (*Opuntia Ficus Indica*) as source of fiber (3% of their energetic requirement) by 30 days [1]. TNF- α and IL-6 were measured in peripheral plasma by commercial ELISA kits. RNA was isolated from peripheral mononuclear cells by Direct-zol RNA MiniPrep. Analysis of relative gene expression of CAT was done by Real-Time Quantitative PCR by $2^{-\Delta\Delta CT}$ method by specific TaqMan probes.

Key Findings: The age of participants was $X = 39.9 \pm 11.1$, IMC at star: $X = 35.1 \pm 4.1$, IMC after intervention: $X = 34.3 \pm 4.5$ ($p = 0.37$). The concentration of TNF- α (star: $X = 11.2 \pm 8.8$ ng/ml. After intervention: $X = 4.6 \pm 4.3$ ng/ml) and IL-6 (star: $X = 20.1 \pm 17.1$ ng/ml. After intervention: $X = 13.9 \pm 12.6$ ng/ml) showed a statistically significant ($p < 0.05$ in both cases) decreased concentration after the intervention. The gene expression of CAT showed an increment of $X = 20.3 \pm 20.8$ after the intervention. Likewise the peripheral concentration of glucose, triglycerides, total-Cholesterol and LDL-Cholesterol decreased significantly ($p < 0.05$) in all cases.

Conclusion: A decreased concentration of peripheral proinflammatory cytokines and an increment in the gene expression of CAT was observed after a dietary intervention high in fiber by means of “nopal” as source of fiber.

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Authorship: César Hernández-Guerrero: Study design. Analysis of data. Document writing.

Alicia Parra-Carriedo: Selection and follow-up of participants.

Carmen Díaz-Gutiérrez: Selection and follow-up of participants.

Silvia Ramírez: Selection and follow-up of participants.

Sylvanna Gordillo: Determination of gene expression of CAT.

Noemí Martínez: Determination of biochemical markers and proinflammatory cytokines.

Keywords: Obesity, proinflammatory cytokines, catalase, gene expression, nopal.

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Complementation with a Standard Nutritional Formula: Beneficial to Nutritional Status and Gastrointestinal Tolerance in Patients with Breast and Cervical Cancer Under Radiotherapy

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Aim: Analyze whether supplementation with a standard nutritional formula improves nutritional status and gastrointestinal tolerance in patients with breast and cervical cancer receiving radiotherapy.

Method: A longitudinal and analytical study in 30 patients (13 cervical cancer and 17 breast cancer), aged 49 ± 11 years. An eating plan plus a standard nutritional supplement (250 Kcal/day with 25% micro and macronutrients) was prescribed during radiotherapy. The weight was evaluated once a week. A 24-hour recall was applied three times a week to determine the average energy intake. The groups were analyzed separately.

Key Findings: Weight loss during treatment, for cervical and breast cancer was 100 to 4500 g. In both groups, there was a significant weight gain (range 0 to 3500 g respectively) ($p < 0.05$). Most had an intake below recommendations (25%) of macro and micronutrients as well as the zinc and vitamin E (below 50%). Nausea (92% and 53%, respectively) were present in both groups; For breast cancer, fatigue in 47%. The gastrointestinal tolerance to standard nutrient formula was adequate.

Conclusion: Participants with breast cancer presented a lower loss with a greater weight gain than with cervical cancer. The complement improved the nutritional status in both groups. Weight loss was 50% lower according to the previous study as well as the improvement of gastrointestinal symptoms.

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Authorship: Rebeca Monroy Torres, University of Guanajuato, Mexico.

Keywords: Breast Cancer, cervical cancer, macronutrients, weightloss, zinc.

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Diet with Antioxidants Ameliorates the Immune Function Impairment in a Mouse Model of Alzheimer's Disease

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The diet is an important factor in the development of Alzheimer Disease. Thus, Mediterranean diet is a protective factor while high fat diet can be negative¹. Antioxidants have been proved useful for improving functions of the immune and nervous systems as well as the neuroimmune communication. Thus, diets with antioxidants improve homeostasis and the health in mice^{2,3}. The aim of the present work was to study the effect of an enriched diet with antioxidants in the immune function of a transgenic mouse model of Alzheimer's Disease (3xTgAD).

Female 3xTgAD mice received a diet supplemented with N-acetylcysteine and tiopronine (0.1%, 3xTg-Diet). Another group of 3xTgAD (3xTgAD-controls) and a group of non-transgenic (NTg) mice received a standard diet. The supplementation started at 2.5 month of age. At 2, 4 and 6 months of age, the peritoneal cells were obtained and the phagocytic capacity of macrophages, natural killer (NK) activity and the proliferation of lymphocytes in basal conditions and in response to the mitogens Lipopolysaccharide (LPS) and Concanavalin A (ConA) were evaluated. Also, the anti-

oxidant glutathione (GSH) and the lipid peroxidation marker malondialdehyde (MDA) were analyzed.

The results showed and impairment in most of the parameters in 3xTgAD and at 4 months, the diet improved the values of the phagocytic capacity, NK activity, GSH and MDA respect to those in 3xTg-controls.

In conclusion, the early impairment of immunity observed in 3xTgAD mice may be neutralized with a diet supplemented with antioxidants.

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Authorship: Noemí Ceprian: this author carry on the experiment, analysed the data, and wrote the article.

Julia Cruces and Antonio Garrido: these authors help with the development of the experiment.

Lydia Gimenez-Llort: this author provided the animals.

Monica De la Fuente: this author designed the study and reviewed the results and the abstract.

Keywords: Antioxidants, immune function, Alzheimer Disease, mice, diet.

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Effect of the Later-Onset of Obesity on Immune Functions of Leukocytes During the Aging Process

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Obesity has been associated with impaired immune defences and chronic levels of inflammation and oxidation. We have previously demonstrated that the onset of obesity during early adulthood is associated with premature immunosenescence, which is aggravated through aging¹. However, the immune effects that the later-onset of obesity during mature age could have on the aging process are not studied yet. The aim of this work was to study several functional and oxidative stress parameters in peritoneal immune cells from mature and old obese female mice. One group of adult female ICR mice were fed with a high-fat diet (60% of fat) during 14 weeks. The other group was fed a standard diet. After that, when mice were mature (42 weeks old) and old (72 weeks old), blood and peritoneal cell suspensions were collected from obese and control groups in order to analyse biochemical parameters, functions and redox state of leukocytes. The results showed that in obese mature mice there is a significant increase in body weight, glucose, triglycerides, cholesterol, basal lymphoproliferation, superoxide anion and glutathione peroxidase levels. In addition, obese mature mice showed a decreased NK activity and lymphoproliferative response to mitogens (ConA and LPS) than non-obese mature controls. With aging, old obese mice continuous to exhibit increased glucose, basal lymphoproliferation and superoxide anion levels as well as decreased lymphoproliferative response to mitogens with respect to non-obese old controls. In conclusion, the later-onset of obesity produce impaired immune response and redox state in mature age, which is partially aggravated with aging.

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Authorship: M.D.F. performed the study design; C.H., I.M.T, B.J. and N.C. conducted the experiments; C.H. performed the data analyses; M.D.F. and C.H. wrote the abstract.

Keywords: Obesity; Immune function; Oxidative stress; Aging.

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The Relationship of Antioxidant Vitamins Intake and Cytokine Profile in In Vitro Response

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Background and Objective: Vitamins A, C and E deficits can lead to alterations in the immune system and cytokine dysregulation. This study was aimed to evaluate the relationship of daily intake of these vitamins and the in vitro effect of tomato extract (TE) addition on cytokine production by peripheral blood mononuclear cells (PBMCs).

Methods: Human PBMCs (n = 7, women's independent samples) were isolated and cultured with PHA and LPS for 48 hs to stimulate cytokine production. Four groups were considered: PBMCs + PHA/LPS + 1, 10 or 20 µM of α-tocopherol from TE (PBMC-1, PBMC-10 and PBMC-20 groups, respectively) and PBMCs + PHA/LPS used as the control group (C). IFN-γ, TNF-α, IL-1β, and IL-10 were measured in culture supernatants by flow-cytometry (CBA, EMD Millipore). The daily vitamin intakes were analyzed from a Food frequency questionnaire. Statistics were performed using SPSS 23. Kolmogorov-Smirnov was applied to assess normality and Pearson correlations were established.

Results: Vitamin A and E intakes were positively correlated with IL-10 in PBMC-1, PBMC-10, PBMC-20 and C group (p = 0.029; p = 0.025; p = 0.013; p = 0.028 and p = 0.008; p = 0.005; p = 0.003; p = 0.006), respectively. TNF-α showed a positive correlation with vitamin C (p = 0.037; p = 0.013) in PBMC-10 and PBMC-20 groups. TNF-α/IL-10 ratio showed a negative correlation with vitamin A and E intake levels (p = 0.033; p = 0.011 and p = 0.045; p = 0.022) in PBMC-1 and PBMC-20 groups, respectively.

Conclusions: Antioxidant vitamin intakes affect the response of PBMCs to mitogens, being involved in the regulation of pro and anti-inflammatory cytokines.

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Authorship: Research question: Maria F Godoy, Ligia E Diaz. Designing the study: Ascension Marcos, Esther Nova, Maria F Godoy.

Carrying it out: Ligia E Diaz, Maria F Godoy.

Analysing the data: Ligia E Diaz, Esther Nova, Maria F Godoy, Nora H Slobodianik, Ascension Marcos.

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Keywords: antioxidant vitamin intake, cytokines, PBMCs, tomato extract.

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Neuroprotective Properties of Withania Somnifera Root Extract in BV2 Microglia Cells

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The current study evaluates the potential of *Withania somnifera*, a traditional Indian herb, to contribute to prevention and progression of neurodegenerative disorders such as Alzheimer's disease through modulation of inflammation and cell protective signalling pathways in lipopolysaccharide (LPS) stimulated BV2 microglia cells, an established neuroinflammation model [1, 2].

BV2 cells were incubated with non-cytotoxic concentrations of *W. somnifera* root extracts (1–100 µg/ml) and stimulated with LPS to elicit a characteristic cytokine response [3] evaluated using real time qPCR, ELISA and Western Blotting methods. Increasing concentrations of *W. somnifera* root extract (25–100 µg/ml) significantly reduced IL-6, IL-1β and iNOS mRNA and secreted IL-6 protein levels. These results, alongside with the lower accumulation of nuclear p65 protein, indicate a dose dependent down-regulation of NFκB signalling, a major redox regulated stress response pathway. Further data demonstrate marked induction of Nrf2 (nuclear factor E2-related factor 2) signalling and increased expression of target genes such as heme oxygenase 1 (HO-1) suggesting enhanced cytoprotective properties through *W. somnifera*. Under the same conditions we found a strong upregulation of TREM2 (<5 fold, from 10 µg/ml), a receptor protein involved in the removal of amyloid beta plaques, one of the main contributors to neurodegenerative disease.

To summarize, our data provide further evidence of multiple cellular effects of *W. somnifera* root extract and its properties against oxidative stress and inflammation on sensitive microglial cells localized to the brain. Our results are of great relevance for the development of diet and supplement based approaches to counteract neurodegenerative and inflammatory disease.

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Authorship: CB formulated the research question and designed the research, LRE and JK performed the experiments and analysed the data. CB wrote the manuscript.

Keywords: Inflammation, *Withania somnifera*, microglia, macrophage, antioxidant.

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Effect of Vitamin D on Adipokines and Cytokines in Mexican Patients with Type 2 Diabetes

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Introduction: Type 2 diabetes mellitus has become a world-wide epidemic, particularly in Mexico. Evidence suggests a link between vitamin D and insulin secreting cells through modulation of inflammatory processes caused by the immune system [1–3].

Objective: To evaluate the effect of 6-month supplementation of vitamin D on adipokines and cytokines of patients with type 2 diabetes mellitus.

Methods: A placebo-controlled cluster-randomized trial of Mexican subjects with type 2 diabetes mellitus. Supplementation consisted of one capsule with 800 IU of Vitamin D3 vs. a placebo capsule filled with cornstarch. Baseline and 24-week anthropometric and biochemical measurements included glucose, insulin, glycosylated hemoglobin (Hb1Ac), leptin, adiponectin, resistin, IL-1B, IL-4, IL-10, IL-8, IL-6, TNF- α , IFN- γ and MCP-1. Data was analysed with paired t-test and repeated measurements ANOVA.

Results: Metabolic control in both groups improved, with statistical significance ($p < 0.05$) in Hb1Ac, Insulin, HOMAb and HOMAIR). Plasma vitamin D decreased significantly with placebo ($t = 3.27$, $p < 0.01$) and increased significantly with supplementation ($t = -5.09$, $p < 0.000$). Most adipokines (adiponectin and leptin) increased during the trial, while all cytokines also increased, with the exception of resistin which showed a highly significant increase in the supplemented group ($t = -7.40$, $p < 0.000$).

Key Findings: Resistin increased with vitamin D supplementation, while metabolic control was adequate.

Conclusions: The role of resistin in the pathophysiology of type 2 diabetes mellitus is still unclear, as well as the effect of vitamin D on adipokine and cytokine production.

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Authorship: All authors participated in the design and development of the project, as well as in the analysis of data and writing of the abstract.

Keywords: Vitamin D, Adipokines, Cytokines, type 2 diabetes Mellitus.

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Effects of N-3 Polyunsaturated Fatty Acids on Proinflammatory Cytokines of Mexicans with Type 2 Diabetes

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Background: There is evidence that inflammation contributes to the development of type 2 diabetes (T2D). Markers of subclinical inflammation such as proinflammatory cytokines, are found to be increased years before the diagnosis of T2D and it is well recognized they play an important role in the pathogenesis of the disease. Studies in humans, animals and cell cultures have provided evidence supporting a beneficial role for n-3 polyunsaturated fatty acids (PUFAs) in conditions associated with inflammation, because n-3 PUFAs can potentially decrease inflammation through several mechanisms, but it is not clear if supplementation can favourably affect biomarkers of inflammation in people with T2D [1–3].

Aim: The purpose of this study was to investigate how n-3 PUFAs affect proinflammatory cytokines in a Mexican T2D population.

Method: Randomized, single-blind, placebo-controlled clinical trial where 54 adults with T2D received either 520 mg of n-3 PUFAs EPA and DHA (PUFA Group) or placebo (PG) daily during 24 weeks. We obtained baseline and final 24 weeks anthropometric and biochemical measurements, including serum proinflammatory cytokines levels.

Key Findings: We found significant reductions in waist circumference ($p = 0.001$) and in serum levels of glucose ($p = 0.011$), IL-6 ($p = 0.000$) and IL-12 ($p = 0.000$) in the PUFA Group. Similar reductions were found in glycosylated haemoglobin ($p < 0.01$), IL-1 β ($p = 0.000$), IL-8 ($p = 0.000$), TNF- α ($p = 0.000$), IFN γ ($p < 0.01$) and MCP-1 ($p = 0.000$) in both groups.

Conclusion: In this study, supplementation with a low dose of n-3 PUFAs during six months improved the inflammatory profile of people with T2D due to a significant reduction in pro-inflammatory cytokines IL-6 and IL-12.

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Authorship: All authors participated in the design and development of the project, as well as in the analysis of data.

Keywords: Type 2 diabetes, proinflammatory cytokines, n-3 PUFAs.

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Associations Between a Mediterranean Diet Pattern and Inflammatory Biomarkers in European Adolescents

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Aim: To test whether the Mediterranean diet score (MDS) and each food-subgroup is associated with inflammatory biomarkers in European adolescents.

Methods: In 464 adolescents (13–17 y) of the European HEL-ENA study, data was available on body composition, inflammation markers, and food intake determined by two computerized 24 h-recalls. The MDS and its food-subgroups (Vegetables, Fruits&Nuts, Pulses, Cereal&Roots, Monounsaturated/Saturated-fat ratio, Dairy, Fish, Meat and Alcohol) were evaluated. A set of inflammation-related biomarkers was measured: IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, TGF β -1, TNF- α , sVCAM-1, sICAM1, sE-selectin, white blood cells, lymphocytes, CD3, CRP, GGT, ALT, and homocysteine. Multivariate and multiple linear regression analyses were adjusted for age, sex, country, socioeconomic status, parental education, adiposity, and smoking habits.

Key Findings: The MDS was associated with CRP(+), and sVCAM-1(–). The subgroups showed the following positive/negative associations: Vegetables with IL-10(+), CRP(+), CD3(+), ALT(+), lymphocytes(+), sE-selectin(–); Fruits&Nuts with IL-4(–), TNF- α (–); Pulses with IL-5(+), IL-6(+), IL-2(–); Cereals&Roots with IL-6(–), IL-10(–); Monounsaturated/Saturated-fat ratio with IL-6(+), TGF β -1(+), sVCAM-1(+boys, girls), homocysteine(–); Dairy with IL-1(+), IL-5(+), IL-6(+), IL-10(+), TGF β -1(+), homocysteine(–); Fish with homocysteine(–); Meat with IL-2(+), IL-10(+); Alcohol with CRP(+), lymphocytes(–). Sex differences were found.

Conclusion: Some specific food-inflammation associations were found, suggesting that diet is to a certain extent already related to inflammation in adolescents and can be used in disease prevention. Also some counterintuitive results were found, which might be due to grouping very different foods into a single group, besides considering that the human body may respond differently depending on the interaction between diet, lifestyle, genetics, biochemical individuality, age and sex.

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Authorship: A.B. Arouca formulated the research question, has analyzed the data and wrote a draft of the paper. N. Michels helped in formulating the research question, analyzing the data and did editing of the first draft. N. Michels and S. De Henauw are co-supervisor and supervisor of A.B. Arouca; L.A. Moreno was coordinator of the HELENA project. All other authors were involved in the HELENA project (coordinator or data collection). A. Marcos was responsible for the inflammatory parameter analyses. I. Huybrechts developed the Mediterranean diet Score. All authors have read the draft and agreed on the final version.

Keywords: Mediterranean diet, inflammation, adolescents, diet-inflammation association.

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Relationship Between Hydration Status and Inflammation Risk in Adolescents. Substudy Up & Down

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Background and Objective: Optimum hydration is essential for a proper functioning of the organism [1, 3]. Inflammatory biomarkers such as C3 and C4 are useful in predicting the risk of cardiovascular diseases and other associated pathologies [2]. The objective of this study was to evaluate the possible relationship between hydration status and inflammation risk in adolescents.

Methods: This is a cross-sectional substudy from the UP & DOWN Study. A total sample of 100 healthy adolescents (60 boys and 40 girls), between 13 and 16 years old, was analyzed. Body composition was measured through Body Mass Index (weight/height²) and Fat percentage by bioimpedance. The inflammatory biomarkers (C3 and C4, in mg/dL) were measured with a blood test, and the hydration parameters [Total Body Water (TBW) and ExtraCellular Water (ECW), in liters] by bioimpedance [1, 4].

Results: 15% of the subjects were below the optimal ECW range, 58% within the ECW range and 27% above this range. None of the adolescents showed a severe degree of dehydration.

When studying the inflammation status taking into account the compliance of the ECW range, adolescents who were below the ECW range showed significantly higher C4 values (25.9 ± 1.8) than those who were within (20.2 ± 0.9) or above the ECW range (20.8 ± 1.3), (p value = 0.027).

Conclusion: Mild subclinical dehydration may affect the inflammatory status in adolescents, therefore, hydration status in this population should be conveniently controlled in order to prevent any inflammation risk.

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Authorship: -JAR-L and SG-M contributed equally to this work. They both planned this substudy as well as the management

and assessment of the adolescents' samples. JAR-L carried out the statistical study. SG-M analysed the biomarkers. AH performed the anthropometric measurements. All authors wrote the abstract. AM is the coordinator of the UP & DOWN Study at the CSIC node and the supervisor of the whole research work as the Immunonutrition Group head.

Keywords: Hydration, Inflammation, Adolescence.

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Lupinus Angustifolius Protein Hydrolysate Reduces Pro-Inflammatory Markers in Healthy Human Volunteers

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Lupine-derived proteins have several positive effects on human health [1]. These effects can be due to the entire proteins or different fragments obtained from digestion in the intestinal tract or during food processing, named hydrolysates [2]. We have shown the in vitro immunomodulatory effects of *Lupinus angustifolius* protein hydrolysate (LPH) obtained by enzymatic hydrolysis [3]. The aim of this study was to evaluate the safety, tolerability and immunomodulatory actions of an experimental functional drink containing the previously described LPH. To that end, we conducted a food trial in 33 healthy volunteers who consumed the experimental drink daily (200 mL; 0.5% LPH) during 4 weeks. Every two weeks, peripheral blood samples were collected and serum and peripheral blood mononuclear cells were isolated to subsequent procedures. The experimental drink was well tolerated using the Likert test and no side effects were reported. Moreover, no modifications in the general parameters of the health status were

observed, analyzed by the SF-36 questionnaire. The drink did not modify any serum biomarkers of hepatic and kidney function. After four weeks of daily ingestion, the beverage managed to reduce the Th1 profile in PBMCs, which are responsible for mediating a pro-inflammatory response. Furthermore, the balance between IL-10 levels, a typical anti-inflammatory mediator, and the production of IFN- γ and TNF (Th1 cytokines) was augmented after 4 weeks of the beverage ingestion. In conclusion, these results show the innocuousness and the efficacy in reducing pro-inflammatory response in healthy human subjects of an experimental drink based on *Lupinus angustifolius* protein hydrolysate.

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Authorship: The following are the authors' contributions: Study design: AC-V, JP, FM, JMG. Study conduct: IC-C, NA-S, AML, FM, JP, MMY, GS-S. Patient recruitment: IC-C, AIA-R, NA-S, AM-L. Data collection: NA-S, IC-C, AIA-R, GS-S. Data analysis and interpretation: IC-C, NA-S, AM-L. Drafting of the abstract: IC-C, AC-V. Approving the final version of the abstract.

Keywords: *Lupinus*, protein hydrolysates, inflammatory response, food clinical trial, peripheral blood mononuclear cells.

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Anti-Inflammatory Effect of *Lupinus Angustifolius* Protein Hydrolysate

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A wide range of studies have underlined that peptides and proteins from foods might have a beneficial biological activity upon human health [1]. These peptides are normally generated from the hydrolysis of food isolate proteins [2]. The aim of the present study was to evaluate the *in vitro* immunomodulatory and antioxidant capacities of a *Lupinus angustifolius* protein hydrolysate (LPH) in primary cultures of human peripheral blood mononuclear cells (PBMCs) from healthy volunteers. Additionally, we studied the beneficial effects of the daily consumption of LPH in mice with experimental autoimmune encephalomyelitis (EAE), an animal model of neuro-inflammation, resembling multiple sclerosis, with the aim of verifying the *in vivo* immunological and antioxidants activities. LPH managed to reduce the PHA-induced production of interferon- γ (IFN- γ), tumor necrosis factor (TNF) and interleukin (IL)-17, classic pro-inflammatory cytokines, quantified by flow cytometry. LPH also increased the mRNA gene expression and the activity of several enzymes implicated in the defense against oxidative stress, such as superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR). Moreover, LPH increased the total antioxidant capacity (TAC) and reduced the proliferative capacity of the PBMCs, without affecting their viability capacity. Finally, the daily treatment with LPH 100 mg/kg significantly reduced the severity of EAE, a pathology characterized by inflammation and oxidative damage [3]. In conclusion, these findings demonstrate that the LPH managed to reduce the pro-inflammatory status and improve the antioxidant defense. Therefore, we can speculate about the biological beneficial effects of functional compound based on this hydrolysate obtained from *Lupinus angustifolius* proteins.

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Authorship: The following are the authors' contributions: Study design: AC-V, JP, FM, JMG. Study conduct: IC-C, NA-S, FM, JP, MMY, GS-S. Data collection: NA-S, IC-C, GS-S. Data analysis and interpretation: IC-C, NA-S, GS-S. Drafting of the abstract: IC-C, AC-V. Approving the final version of the abstract.

Keywords: Lupine protein hydrolysate, inflammatory response, oxidative stress, human lymphocytes, multiple sclerosis.

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Vitamin D Uptake in Overweight and Obese Occupational Asthma Patients

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Objective: Overweight and obese patients are generally considered at risk of vitamin D deficiency. For its known actions in modulation of the immune function and inflammation reduction, vitamin D deficiency could aggravate asthma evolution. We aimed to assess the vitamin D intake in occupational asthma overweight and obese patients.

Population: 15 patients with mild to moderate form of asthma that visited the clinic for their annual medical control during February 2017 were recruited.

Methods: Diet vitamin D intake was assessed with a validated questionnaire for the Romanian population. None of the patients had vitamin supplements intake. Current health status perception was evaluated using the Saint George questionnaire. Basic statistics was computed using StatPlus: macPro version v6.

Results: The average age was 52.85 years and the average BMI was 31.85. 14 out of the 15 patients had normal FEF1 and FEV1/CV values, one had mild obstruction and 5 presented with a distal obstruction (MEF50 <40%). The average number of days/week without symptoms was 2.5.

All patients had vitamin D intake lower than RDI. We calculated an average vitamin D intake of 227.17 UI, with a wide variation coefficient (53%). Prevalence of obesity associated disorders (hypertension, dyslipidemia, coronary heart disease and diabetes) was very high (86%).

Conclusions: Vitamin D intake in overweight and obese occupational asthma is below RDI in all patients, possibly due to the recommendation for a low lipid intake imposed by the obesity associated diseases. Co-morbidities related diet reduces vitamin D uptake in overweight and obese occupational asthma patients.

Authorship: Both authors were involved in the design of the study. Agripina Raşcu conducted the patient investigation. Marina Otelea processed the data and wrote the abstract.

Keywords: Vitamin D, occupational asthma, obesity, lung function.

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Inflammatory Biomarkers and Obesity in Youth with and Without Down Syndrome

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Background and Objective: Inflammation has been related to obesity and its associated pathologies, such as cardiovascular diseases and metabolism alterations. Adipose tissue excess leads to elevated inflammatory biomarkers, although there is still a lack of knowledge on children and adolescents, particularly in a very special group such as subjects with Down syndrome (DS). This study was aimed to evaluate possible associations between inflammatory biomarkers and obesity in a sample of children and adolescents with DS and without DS (controls).

Methods: A total of 502 children and adolescents (101 with DS) aged 10–20 years-old, were recruited in this UP&DOWN sub-study. Insulin, leptin, TNF- α , adiponectin, galactain3, visfatin and cortisol were selected as inflammatory biomarkers. Height and weight were measured and Body Mass Index (BMI) was calculated. Pearson correlations were executed.

Results: 65.3% and 31.9% of the volunteers had values below the level of detection in the visfatin (0.156 ng/ml) and insulin (1.00 pg/ml) test respectively. BMI was higher in DS than in controls ($p < 0.001$). Galactin-3 and adiponectin levels were lower in DS than in controls ($p < 0.001$). BMI was positively correlated with leptin and insulin and negatively with adiponectin in both groups. On the other hand, BMI positively correlated with TNF- α and cor-

tisol in the control group and negatively with visfatin in the DS group (all $p < 0.05$).

Conclusions: These results point out that children and adolescents with SD have a peculiar inflammatory behaviour. An exhaustive study of the relationships between body composition and inflammatory biomarkers should be further evaluated in this group of population.

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Authorship: Ana Gutiérrez-Hervás^{1-2*}, Sonia Gómez-Martínez^{2*}, Ana María Veses², Rocío Izquierdo-Gómez³, Óscar Veiga⁴, Álvaro Delgado-Alfonso⁵, José Castro-Piñero⁵, Catalina Santiago⁶, Félix Gómez-Gallego⁶, Ascensión Marcos².

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*Ana Gutiérrez-Hervás and Sonia Gómez-Martínez contributed equally to this work.

Keywords: inflammatory biomarkers; children and adolescents; Down syndrome.

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Effect of *Lactobacillus Rhamnosus* GG on Immune Functions of Prematurely Aging Mice

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The immune system is particularly affected during the aging process, with several aged-related changes in its functions. This immunosenescence results in a higher risk of morbidity and mortality [1]. The supplementation with probiotics could be a good nutritional strategy to improve immune functions of aged individuals. This study evaluated the effect of the supplementation with *Lactobacillus rhamnosus* GG on two relevant immune functions of peritoneal leukocytes in a model of premature aging mice. Adult mice based on an altered stress-related behavioral response were divided into two groups; one group of prematurely aging mice (PAM) ($n = 9$) and the other of non-prematurely aging mice (NPAM) ($n = 9$). PAM and NPAM were then supplemented with *L. rhamnosus* GG. Controls without probiotic intake were also analyzed. After 2 weeks of treatment, natural killer (NK) activity against tumor cells and proliferative response to the mitogens ConA and LPS were analyzed in peritoneal leukocytes. The results showed that the NK activity increased after supplementation with *L. rhamnosus* GG in PAM ($P < 0.01$) with respect to control mice. In addition, supplemented PAM exhibited higher NK activity than NPAM ($P < 0.05$). There also was an improvement in the proliferative response in leukocytes of PAM, especially with ConA. These results show that *L. rhamnosus* GG supplementation in a short period of time results in an improvement of two immune functions in adult mice with premature immunosenescence. Since these functions are markers of health, we suggest that this probiotic may be useful for achieving a healthy aging.

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Authorship: M.D.F. and T. R. contribute to the study design; P.H. and C.H. conducted the experiments; P.H. performed the data analyses; M.D.F., P.H. and C.H. wrote the abstract.

Keywords: Probiotics, Immune function, Ageing.

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NK Activity in Spleen Cells of Rats After Exhausting Exercise

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It is established that an intense and exhaustive exercise induces adverse effects on the immune system [1] and, among other, it has been reported in humans a decrease in the activity of NK cells [2, 3]. In this situation, nutrition may play a key role by preventing or reducing the immunodepression. The aim of this study was to establish spleen NK activity after an exhausting exercise in rats in order to reflect the depression of the immune function.

After 4 weeks of training in a treadmill, young Wistar rats were induced to exercise until extenuation. Immediately after the fatigue test, animals were anaesthetized and exsanguinated. Spleen lymphocytes were isolated and evaluated their composition by flow cytometry. In addition, the cytotoxic activity of NK cells was assessed by flow cytometry using K562 cells as target cells.

In the spleen, runner rats showed higher proportion of NK cells than sedentary rats. However, the cytotoxic activity of these cells of runner rats decreased significantly.

In conclusion, after the 4 weeks of training and a session of exhausting exercise, runner rats showed a reduction in NK cytotoxic activity. This could constitute a biomarker of immunodepression to be prevented by nutritive complements.

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Authorship: M.C., F.P.-C. and A.F. designed the study, S.E.A., M.C.-B. and M.P. carried it out, S.E.A. analysed the data, M.C. and S.E.A. wrote the abstract.

Keywords: Exhausting exercise, lymphocytes, NK activity, spleen.

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Nutritional Intervention Based on Mediterranean Diet Reduces Blood Pressure and Immune Cells Response After Immune Stimulation

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Metabolic syndrome and obesity are main health problems of modern life in the twenty-first century [1]. Low-grade inflammation is directly associated with a cluster of disorders collectively known as the metabolic syndrome [2]. The aim of the study was to determine the effects of a nutritional intervention based on Mediterranean Diet on peripheral blood mononuclear cells (PBMCs) and neutrophils capabilities to produce reactive oxygen species (ROS) after immune stimulation. Subjects aged 55 to 75 years, with overweight/obesity and metabolic syndrome were included in the study. Subjects were divided in two groups: 1) Intensive lifestyle intervention with a hypocaloric Mediterranean diet, physical activity and behavioural therapy and 2) a control group assigned to non-intensive advice on healthy diet (also Mediterranean type) following usual clinical practice. Anthropometrical and blood samples were obtained at the beginning and after 6 months follow-up. No significant differences were reported in the body mass index, glucose, lipid profile or in markers of tissue damage (AST, ALT and GGT) after the 6th month of intervention. Systolic blood pressure was significantly reduced in both groups. ROS production decreased in both PBMCs and neutrophils after 6 months of intervention when stimulated with zymosan and lipopolysaccharide, without significant differences between groups. Although both dietary interventions reduced the production of ROS by immune cells, the analysis of a larger number of subjects and over a longer follow-up period is necessary to determine the efficacy of the most intensive intervention.

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Authorship: Antoni Sureda: carrying the study, analysing the data and writing the article.

Carla Busquets-Cortés, Xavier Capó, Emma Argelich, Alejandra Arenas, David Mateos: carrying the study.

Antoni Pons, Josep A Tur: designing the study, analysing the data and writing the article.

Keywords: Inflammation, immune cells, Mediterranean diet, metabolic syndrome.

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Diet Supplementation with Docosahexanoic Acid Attenuates the Inflammatory Response to Exercise of Immune-Stimulated Peripheral Mononuclear Cells

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The practice of regular physical exercise has several health benefits. Polyunsaturated omega-3 fatty acids contribute to regulate inflammation derived from their anti-inflammatory effects [1, 2]. The aim was to investigate the effect of 8-weeks docosahexanoic acid (DHA) diet supplementation on the peripheral mononuclear (PBMCs) cells cytokine production after an acute bout of exercise. Fifteen male football players were randomly assigned to a placebo or an experimental group consuming a functional beverage enriched with DHA (1.16 g/day). The DHA content increased in erythrocytes after 8 weeks of training in the supplemented group. Neither diet supplementation with DHA nor training season altered the basal plasma cytokines, and acute exercise only increased plasma IL6 in both experimental and placebo groups. Ex vivo lipopolysaccharide (LPS) stimulation of PBMCs induced an inflammatory response with a significant production rate of TNF-alpha, IL6 and IL8 after acute exercise. The supplementation with DHA significantly reduced the production of TNF-alpha and IL6 in stimulated PBMCs. Acute exercise significantly increased the reactive oxygen species (ROS) production in both groups, although this increase was significantly higher in the placebo than in the experimental group. The production rate of resolvin D1, a pro-resolving mediator of inflammation, by LPS-stimulated PBMCs increased after acute exercise in both groups, but the increase was only significant in the experimental group. In conclusion, exercise increased the PBMCs cell capability to produce cytokines after stimulation with LPS and this cytokine production was attenuated by diet DHA supplementation.

Financial Support: Acción Estratégica en Salud del Ministro de Ciencia e Innovación (Projects 11/01791, PI14/00636, CIBERObn CB12/03/30038), FEDER Funds, COST Action CA16112.

Authorship: Silvia Tejada: analysing the data and writing the article.

Antoni Sureda: carrying the study, analysing the data.
Carla Busquets-Cortés, Xavier Capó, Miquel Martorell, Juan M. Batle, Isabel Llompart: carrying the study.
Josep A Tur, Antoni Pons: designing the study, analysing the data and writing the article.

Keywords: exercise, inflammation, immune cells, omega-3 fatty acids.

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Effect of Different Amounts of Alcohol Intake on the Gut Microbiota Composition in Healthy Adults from Two European Countries

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Background and Objective: Knowledge on the effect of alcohol consumption on the gut microbiota is scarce, and seems to be different depending of the amount of alcohol intake. Thus, this work aims to assess the effect of the amount of alcohol intake on the microbiota from healthy adults.

Methods: 261 Spanish (25–45 y) (51% males, 49% females) and 229 Slovenians (42% males, 58% females) healthy adults were studied. Volunteers' alcohol consumption habits were analysed by using a validated questionnaire, and classified according to their alcohol consumption: 1) None-Low (N = 124 and N = 95): <5 g alcohol/d; 2) Moderate: between ≥5 g/d and ≤20 g/d (men) or ≤12 g/d (women) (N = 99 and N = 67); and 3) High: >20 g/d (men) or >12 g/d (women) (N = 38 and N = 67). Gut microbiota was analyzed through 16S rRNA gene amplicon sequencing (V3+V4 gene regions. MiSeq 2x250 Illumina) and taxonomic analysis. General lineal models and Kruskal-Wallis plus Mann-Whitney tests were performed.

Results: Gender differences were observed in both countries. Women showed higher levels of Firmicutes (P = 0.001) and lower levels of Bacteroidetes families than men. The High group showed higher levels of Ruminococcaceae (P = 0.013) in Slovenians, and Fusobacteriaceae (P = 0.028) and Spirochaetes (P = 0.001) in Spanish than Moderate and None-Low. Other families differences varied between men and women in each country.

Conclusions: The amount of alcohol intake affects the microbiota composition in a different way depending on sex and country, thus suggesting that not only sex but also environmental factors should be considered to disentangle the influence of alcohol consumption on the microbiota from healthy adults.

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Financial Support: European Foundation for Alcohol Research (ERAB).

Authorship: Noemí Redondo¹, MSc; Brenda Villavisencio¹, BSc; Ligia E. Díaz¹, PhD; Ascensión Marcos¹, PhD; Esther Nova¹, PhD.

Keywords: Gut microbiota, alcohol intake, 16S metagenomics, healthy adults.

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Plasma Phosphatidylcholine Saturated and Monounsaturated Fatty Acids in Nigerian and Mexico Type 2 Diabetes Mellitus Patients

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Background: Saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) are associated with DM2 [1–3], but no research has been done to evaluate this in Nigerian diabetics compared to other populations.

Objective: Compare SFAs and MUFAs in Nigerian and Mexican DM2 subjects.

Methods: Nigerian (DM2; n = 68, control; n = 33) and Mexican (DM2; n = 55, control; n = 50) subjects fasting plasma were analysed for phospholipid (PC) fatty acids by GLC, and mean % fatty acids compared in diabetics and controls, $p < 0.05$.

Results: Palmitic acid; C16:0 was higher in DM2 subjects of both populations (Nigeria; 30.83 ± 3.86 vs. 29.04 ± 4.22 , Mexico; 30.96 ± 2.45 vs. 29.48 ± 2.20), and this FA has been linked to inflammation and DM2 [1]. Stearic acid; C18:0 was lower in DM2 (Nigeria; 15.88 ± 2.21 vs. 17.57 ± 2.43 , Mexico; 13.76 ± 1.88 vs. 14.99 ± 1.20), a pattern reported in another study [3] but not in others [2]. Arachidic acid; C20:0 was different only in Mexicans (Controls; 0.08 ± 0.06 , DM2; 0.05 ± 0.05). Vaccenic acid; C18:1n-7 was the only MUFA different in the populations and was higher in Nigeria controls; 1.26 ± 0.32 vs. DM2; 1.05 ± 0.39 . C18:1-n7 has been reported to be associated with reduced incidence of DM2 [1, 3].

Discussion: This study further supports the association of C16:0 with DM2, but not C18:0 in Nigerian and Mexican DM2 subjects. The similar SFAs and MUFAs pattern in both populations in relation to DM2 needs to be further investigated.

Acknowledgments: We thank the clinicians and nurses who helped us obtain blood samples.

Authorship: All authors participated in the design and operational aspects of the study.

Keywords: Nigerian Type 2 diabetes mellitus, Mexican type 2 diabetes mellitus, plasma saturated fatty acids, plasma monounsaturated fatty acids.

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Circulating Cytokines and Phospholipid Fatty Acids in African Nigerian Type 2 Diabetes

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Background: Inflammation contributes to the development of type 2 diabetes mellitus (DM2) [1] and we reported elevated IL-6 in Nigerian DM2 [2]. Polyunsaturated fatty acids (PUFAs) affect inflammation and cytokines [3].

Aim: Plasma phospholipid fatty acids and cytokines in Nigerian DM2 and age matched control population.

Methods: DM2 (n = 68) and control (n = 33) plasma analysed for phospholipid (PC) fatty acids and cytokines by GLC and Luminex multiple analyte profiling.

Results: Palmitic acid positive relationship with resistin ($r = 0.302$, $p = 0.026$); stearic acid positive correlation with TNF- α ($r = 0.322$, $p = 0.021$) and IL-6 ($r = 0.286$, $p = 0.047$) in DM2. Linoleic acid negative relationships with TGF- β ($r = -0.299$, $p = 0.028$) and MCP-1 ($r = -0.321$, $p = 0.018$) and AA/DGLA with TNF- α ($r = -0.365$, $p = 0.008$) negative correlation in DM2. Positive correlations between EPA and TGF- β ($r = 0.293$, $p = 0.031$) and DHA with TGF- β ($r = 0.285$, $p = 0.037$), and IL-12 ($r = 0.302$, $p = 0.030$) in DM2. Controls positive correlation AA and leptin ($r = 0.477$, $p = 0.012$), total n-6 PUFAs and leptin ($r = 0.422$, $p = 0.032$), total n-6 PUFAs negative correlation with IL-6 ($r = -0.395$, $p = 0.046$) and

total n-6 and n-3 PUFAs positive relationships with leptin ($r = 0.447$, $p = 0.022$) and IL-4 ($r = 0.521$, $p = 0.008$).

Conclusion: Nigerian DM2 and healthy population have weak to moderate correlations between fatty acids and cytokines.

Acknowledgments: We thank the clinicians and nurses who helped us obtain blood samples.

Authorship: All authors participated in the design and operational aspects of the study.

Keywords: Nigerian Type 2 diabetes mellitus, plasma n-6 PUFA, n-3 PUFA, cytokines.

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Efficacy of Vaccination with *Staphylococcus Aureus* Enterotoxin B in Senescent Mice

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Aging is characterized by an increased susceptibility to infections and a reduced capacity to tolerate innocuous antigens that compromise the protective functions of the gut mucosa. The present study evaluated the immune response to nasal vaccination with *Staphylococcus aureus* enterotoxin B (SEB) in a mice model of senescence. Mice vaccinated with low SEB doses and non-vaccinated mice were exposed to a lethal shock induced by SEB. Experiments were performed on young (2 months-old) and old (6 months-old) SAMP8 mice showing an accelerated senescence profile. Mice received nasal doses of 2.5 µg of SEB (vaccinated group) or vehicle (controls) every 12 days (3 times). Fifteen days after the last dose, mice were challenged with i.p. SEB and LPS (10 µg and 75 µg, respectively). Vaccination did not modify the fecal and broncho-alveolar concentration of total IgA, but vaccinated mice showed high titers of anti-SEB IgA in both samples ($P < 0.05$). Lethal SEB doses reduced the central temperature in non-vaccinated mice and this effect was higher in senescent than in young mice ($P < 0.05$). Only 9% of non-vaccinated senescent mice survived, while 90% of vaccinated senescent mice survived. Young mice were more resistant to the SEB challenge since 50% of non-vaccinated survived compared to the 100% survival in young vaccinated one. Death induced by lethal shock was preceded by a drastic fall on central temperature, indicating that this variable is a valuable

marker of mice response. Vaccination improved the survival in young and old mice which was especially relevant in senescent mice.

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Conflict of Interest: A.P.-B., L.M., AG.-J., C.A. and M.M. declare no conflict of interest. J.P. is employed by APC Europe SLU (Granollers, Spain.). The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data.

Authorship: A.P.-B., C.A., J.P. and M.M. conceived and designed the experiments;

A.P.-B., L.M. and A.G.-J. performed the experiments;

A.P.-B., L.M. and M.M. analyzed the data;

J.P. provided the experimental diets;

A.P.-B., L.M. and M.M. wrote the communication.

All the authors approved the final version of the communication.

Keywords: SAMP8, aging, SEB, vaccine, Immunoglobulin A.

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Effects of Chia Oil-Enriched Restructured Pork on Inflammatory Markers of Aged Rats in Nonalcoholic Steatohepatitis Model

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Chia oil has the highest recognized α -linolenic acid (ALA) content. ALA is associated with the prevention of cardiovascular diseases, mainly related with saturated fatty acid metabolism. Present work aims to analyze the effect of Chia oil-enriched restructured pork (RP) on aged rats in a nonalcoholic steatohepatitis (NASH) model. Groups of six male Wistar rats (1-year old) were fed three experimental diets containing RP formulated with saturated fat added or not with pork fat or chia oil: high-saturated fat control RP diet (C); cholesterol-enriched high-saturated fat/high-cholesterol control RP diet (HC); and Chia oil-RP cholesterol-enriched high-saturated fat/high cholesterol (CHIA). TNF α , iNOS, eNOS protein expression and inflammatory index were determined. TNF α results appear to be relevant, as the increase observed in this inflammation marker following the HC diet (80% higher than C)

was partially blocked by CHIA diet, even 10% less than C. However, surprisingly, both CHIA and HC induced a reduction of 10% in eNOS/iNOS ratio compared to C. Results suggest that the anti-inflammatory activity induced by CHIA-RP in aged rat was dependent of TNF α expression but independent of the NOS-system stressing the importance of incorporating ALA into the RP and diet. More studies are needed to confirm present results.

Keywords: Ageing; Chia oil; NASH, inflammation; restructured pork.

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In Vivo Study on the Possible Anti-Inflammatory Properties of Non-Alcoholic Beer and Associated Compounds

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Aluminum (Al) has long been implicated in the pathogenesis of Alzheimer's disease. It appears that the Al interacts with the cholinergic system, altering the functioning of the cholinergic projection and intensifying the inflammation. The present paper aims to study the capacity of two compounds, silicon and hop-rich in polyphenols-, contained in non-alcoholic beer (NAB) to reduce the inflammation of Al in rats. Al was long-term administered to male Wistar rats through drinking water at a dose of 450 μ g/mL for 3 months. Others groups were given Al + non-alcoholic beer (5 mL); Al + hops solution (0.4 mg/mL); and Al + silicic acid solution (Si(OH)₄) (50 mg/L). A negative control group received water instead. Results showed that Al intoxication induced an increase in TNF α levels and expression of IL1 β in rat brains. The administration of NAB significantly decreased these parameters, although by different and complementary ways: a) the detoxification of Al, possibly by forming non-toxic aluminosilicate, and b) the presence of polyphenols (e.g. xanthohumol) in hops. Therefore, results permit concludes that moderate consumption of NAB exerts a prophylactic effect alleviating the inflammatory effects induced by Al poisoning in brain.

Keywords: Aluminium, hops, inflammation, neurotoxicity, non-alcoholic beer, silicon.

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The Consumption of Reduced Fat-Pork Products Decreased Body Fat and TNF α in Volunteers at Increased Cardiovascular Risk. A Preliminary Study

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High meat-product consumption has been related to cardiovascular disease (CVD). However, previous results suggest the benefits of consuming reduce fat meat products on lipoproteins. Present study aims to assess the effect of consuming reduced fat-Pâté and Frankfurter on some anthropometric and inflammatory markers (e.g. body fat and tumor necrotic factor- α (TNF α), respectively) in male volunteers at increased CVD risk. Eighteen male volunteers with at least two CVD risk factors were enrolled in a cross-over controlled study. These report shows preliminary data obtained through the first phase of the study where volunteers consumed reduced fat pork-products (RF). Looking for specific target, the effects were also tested among volunteers with different initial LDL-cholesterol levels (< and $\geq$ 3.36 mmol/L). RF products decreased TNF α 25.6% ($p < 0.01$) and the body fat mass 2% ($p < 0.05$) in all volunteers. In volunteers with low initial LDL-cholesterol levels this reduction was significant for TNF α (27%, $p < 0.05$) and body fat mass (4.8%; $p < 0.05$). Results suggest the benefits of consuming RF products; however, the effects on body fat were small and belong to a small sample of volunteers. More studies are needed to clarify mechanisms involved in order to ascertain "targets" subjects.

Keywords: Body fat, inflammation, LDL-cholesterol; Obesity; Reduced fat-pork products; TNF.

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The Impact of Trypanosoma Cruzi Infection on the Evolution of Cardiovascular and Liver Disease Induced by Fat Diet: The Significance of Toll Like Receptor-4

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Obesity is considered an epidemic inflammatory disease with great impact on human health. The metabolic syndrome (MS) is associated with cardiovascular and liver disease. Previously we developed a MS model in C57BL/6 WT fed with fat diet after 4 months [1].

Objective: To elucidate the effect of T. cruzi infection in the pathogenesis of MS and investigate the role of TLR4 innate recep-

tor in this process. Thus, male C57BL/6 mice WT and TLR4-/- mice were divided into 4 groups B, basal with standard feed (3% fat), D, 13% fat diet, I (100 T. cruzi parasites-Tulahuén) and I+D, Infected+Diet.

Results: There was an increase in body weight in D-WT vs. B ($p < 0.001$). Besides, this group showed adipocyte hypertrophy in aortic adventitia and lipid micro/macrovésículas and inflammatory foci compatible with steatohepatitis, not observable in TLR4-/- . Notably, the groups I+D (WT and KO) didn't show ectopic fat but showed an increased number of liver leukocytes. A significant increase of CD36 expression was detected in the liver and aorta only in D-WT. F4/80+CD68+CD206- cells were observed in liver of animals D and I+D vs. B only in WT ($p < 0.05$). CD8+ T cells were increased in D-WT group. The % of CD8+IFN γ + cells was increased in group D-WT vs. B. There was also an increase in the % of CD4+ and CD8+ in I+D-WT.

Conclusion: These results clearly demonstrate the involvement of TLR4 receptor in the model of MS, and parasite infection improves steatohepatitis and lipid deposition in the aorta but increases the number of inflammatory cells.

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Authorship: LO SG. Performed the experiments: LO AA AP RC. Analyzed the data: LO AA SG Contributed reagents/materials/analysis tools: SG.

Keywords: cardiovascular disease, fat diet, infection, Toll like receptor-4.

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Sleep Disorders in Overweight/Obese Children

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Objective: To analyse sleep disorders in overweight/obese children and adolescents.

Material and Method: This descriptive, prospective study was conducted with a sample of 45 obese children in Granada (Spain) from 2013 to 2016. A polygraphy was performed to determine the existence or otherwise of sleep disorders. The abbreviated Spanish version of the Paediatric Sleep Questionnaire (PSQ) was also applied.

Results: 62% of the children assessed had sleep disorders. Of those with sleep apnea-hypopnea syndrome (SAHS), 56% had a pathological desaturation index and 44% had a pathological grade of apnea-hypopnea.

Conclusions: This study revealed a strong relationship between overweight/obesity and sleep disorders in children. Accordingly, polygraphy is suggested as a means of obtaining an accurate diagnosis of SAHS. Moreover, an intervention programme should be undertaken to reduce overweight and obesity, and consequently to improve the quality of sleep in children and adolescents, and benefit their families. Also, obesity reduces immunology in children.

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Authorship: María José Aguilar wrote the final draft of the manuscript.

Antonio Manuel Sánchez and Ignacio Valverde recruited the participants and administered the questionnaire.

Tania Rivero contributed the translation into English.

Tomás Muñoz performed the respiratory polygraphy tests at the hospital.

All authors have reviewed the abstract.

Keywords: Sleep apnea, childhood obesity, respiratory polygraphy.

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Immunological Characteristics of Breast Milk

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Objective: To analyse the nutritional composition and immunological properties of human milk according to the characteristics of the lactating woman.

Material and Method: This descriptive, prospective study was conducted with a sample of 100 lactating women, divided into two groups: healthy and at risk. The study included the analysis of milk from women who had preterm infants and those who were of Roma ethnicity.

Results: The quantitative analysis of human milk from different groups of women revealed differences in lipid profiles. The Roma women presented significant differences from the other participants for some immunological factors.

Conclusions: Babies receiving breast milk are less liable to suffer from gastrointestinal, respiratory, allergic or asthma diseases, and childhood obesity. In addition, breast milk decreases the risk of breast cancer, cardiovascular disease and metabolic syndrome in adult life. Exclusive breastfeeding until at least 6 months of life should be encouraged.

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Authorship: Noelia Barea, Laura Baena and M^a Dolores Moraleda contributed with the literature search and analysis of the results. Tania Rivero contributed the translation into English.

María José Aguilar and Antonio Sánchez wrote the abstract.

All authors have reviewed the abstract.

Keywords: Breastfeeding, immunological factors, lipid profiles.

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Supplementation with *Lactobacillus Fermentum* D3 During Pregnancy and Its Influence on Infant Intestinal Microbiota

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Aims: The overall aim of this project is to determine the degree of colonisation of beneficial bacteria in the microbiota of the pregnant woman and her baby, by administering to the mother a lactic food supplement based on *Lactobacillus fermentum* D3. The specific aims are:

- a) To assess the nutritional status of pregnant women.
- b) To identify the characteristics of the maternal microbiota, before and after the probiotic intake.
- c) To assess the influence of the probiotic intake on the characteristics and composition of breast milk.
- d) To identify the main bacterial strains established in the microbiota.
- e) To assess the beneficial effects (quantitative and qualitative) of probiotic supplementation in the mother and in the baby.

Method: A prospective cohort study will be conducted in women during pregnancy and lactation, as well as in their postpartum babies. A total sample of 120 healthy women will be recruited, in collaboration with the Hospital Complex of Granada. The participants will be divided into two groups, both composed of healthy women. Personal and family history will be recorded for each group, by means of a detailed clinical history and anthropometric and nutritional assessment, in order to take into account all the variables that may influence the level of intestinal microbiota (including type of diet and intake of the food supplement). DNA extraction, sequencing and bioinformatic analysis, and the isolation and quantification of *Lactobacillus* and *Bifidobacteria*, will be performed.

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Authorship: María José Aguilar and Manuel Olalla wrote the final draft of the manuscript.

Laura Baena, Aurora Bellido and Rafael Giménez recruited the participants and coordinated the collection of human milk.

Antonio Manuel Sánchez contributed the translation into English.

All authors have reviewed the abstract.

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Chia Oil-Enriched Restructured Pork Consumption Decreases Liver Inflammation in Aged Rats

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Oil extracted from the *Salvia hispanica* L. seeds, known as chia oil, is very rich in n-3 polyunsaturated fatty acids (PUFAs), especially α -linolenic acid. n-3 PUFAs decrease cytokines, inflammatory eicosanoids, and reactive oxygen species, and hence have been suggested to be an anti-inflammatory agent. Restructured pork (RP) has been found to be valuable matrix to include functional ingredients. The present study aims to assess the effect of diets containing Chia oil-RP on the liver inflammation in a nonalcoholic steatohepatitis (NASH) model assessed in aged Wistar rats. Degree of evolution of non-alcoholic fatty liver disease (NAFLD) to NASH was histopathological evaluated according to a specific score. One-year-old rats were fed with three experimental diets containing RP: a) a high saturated fat diet as a NAFLD control (C); b) high saturated/high cholesterol diet (HSHCD) as a model of NASH (Chol-C); and c) the HSHCD with Chia oil-RP (Chol-Chia). In comparison with Chol-C diet, Chol-Chia diet significantly decreased portal and lobular inflammation and hepatocellular ballooning close to those C rats. Chia oil-RP significantly arrested inflammation associated to NASH. Present results suggest that Chia oil-RP consumption induces liver anti-inflammatory effects acting as a functional ingredient when incorporated to meat derivative.

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