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Proceedings of the Fourth Belgian Nutrition Society Symposium 2014: Genes and nutrition, is personalised nutrition the next realistic step?

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INTRODUCTIONS

I1

Genes and nutrition, is personalised nutrition the next realistic step

Christophe Matthys[1*], Stefaan De Henauw[2], Patrick Kolsteren[3], Carl Lachat[4], John Van Camp[4], Kristin Verbeke[5], Nathalie Delzenne[6]
[1]Clinical and Experimental Endocrinology, KU Leuven, B-3000 Leuven, Belgium; [2]Department of Public Health, Ghent University, B-9000 Ghent, Belgium; [3]Unit of Nutrition and Child Health, Institute for Tropical Medicine, B-2000 Antwerp, Belgium; [4]Department of food safety and food quality, Ghent University, B-9000 Ghent, Belgium; [5]Translational Research in GastroIntestinal Disorders, KU Leuven, B-3000 Leuven, Belgium; [6]Louvain Drug Research Institute, Metabolism and Nutrition Research Group, Université catholique de Louvain, B-1040 Brussels, Belgium
E-mail: christophe.matthys@uzleuven.be
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Early 2014, the US Academy of Nutrition and Dietetics wrote in its position statement that "nutritional genomics provides insight into how diet and genotype interactions affect phenotype"[1] Nutrients can dictate phenotypic expression of an individual's genotype by influencing the processes of gene transcription and protranscriptional processes (including translation). More important, the US Academy identified the practical application of nutritional genomics in the management of complex chronic disease as an emerging science [1]. Nutritional genomics is often presented as the new 'holy grail' in nutrition with an ultimate goal to establish a so-called personalised nutrition – i.e. an individual diet tailored to genotype-driven needs. However, one could wonder what the current state of the art is of this concept and to what extent it is realistic to expect such achievements in the near future.

During the fourth Belgian Nutrition Society Symposium the different aspects of the broad field of personalised nutrition have been discussed. New evidence of the importance of diet through the life-course is coming from epigenetics, i.e. changes in the regulation of the expression of gene activity without alteration of genetic structure [2]. There is now considerable evidence for nutritional epigenetic programming of biological functions. Impaired programming has been related to a wide range of phenotypes including obesity and diabetes. Prof Cnop covered in her talk the role of epigenetics in the development of obesity and diabetes.

A nutritionally relevant Single Nucleotide Polymorphism (SNP) is the C677T polymorphism. It is a common SNP of the methylenetetrahydrofolate reductase (MTHFR) gene, which encodes for the 5,10-MTHFR enzyme and uses folate to metabolize and thereby remove homocysteine. As homocysteine increase is considered a risk factor of cardiovascular diseases, the nutritional importance of this SNP is clear. Prof Helene McNulty has discussed in her talk the role of MTHFR, riboflavin and hypertension based on new and recently published data.

In December 2013, Science's editors announced that research regarding the role of bacteria living inside the human body and their vital roles in determining how the body responds to challenges as malnutrition or cancer was one of the "Breakthroughs of the Year" [3]. In the same time period a letter in Nature was published which indicated the importance of the composition of the gut microbiota to explain the response towards nutritional intervention in obese individual [4,5]. In 2011, our annual meeting had been fully devoted to the relationship between the gut microbiota, nutrition and health (abstracts available at http://www.belgiannutritionsociety.be/data/userfiles/File/BNS2011-abstract-book.pdf). This year, Prof Jeroen Raes discussed in more depth the importance of the genome and the microbiome in human well-being.

Although the US Academy of Nutrition and Dietetics announced the practical application of nutritional genomics for complex chronic disease as an emerging science, it has recently been argued that personalised nutrition will not have the dramatic impact that was once expected [6]. Before personalised nutrition is part of the daily clinical practice further understanding of the complex influences of genetics and the interaction with diet is necessary. Nevertheless Prof Anne-Marie Minihane presented in detail the translational aspects of nutrigenomics. Finally Jo Goossens has discussed the possibilities of nutrigenomics from a business point of view.

This session has been assorted of oral presentations presented upon selection of abstracts, putting forward the dynamism of our young researchers in the field of nutrition and health in our country.

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KEYNOTE LECTURE PRESENTATIONS

K1

Epigenetic aspects of pancreatic beta cell function in type 2 diabetes

Miriam Cnop
Laboratory of Experimental Medicine, Division of Endocrinology, Erasmus Hospital, ULB Center for Diabetes Research, Universite Libre de Bruxelles, Brussels, Belgium
E-mail: mcnop@ulb.ac.be
Archives of Public Health 2014, **72(Suppl 1):**K1

Genetic and environmental factors contribute to the pathogenesis of type 2 diabetes. Epigenetic changes link environmental exposures with potentially heritable disease mechanisms. Pancreatic beta cell failure is central in the pathogenesis of type 2 diabetes. The gold standard for elucidating the underlying mechanisms is the study of human islets of Langerhans. We performed the first comprehensive DNA methylation profiling of islets from type 2 diabetic and non-diabetic donors [1]. We identified differential DNA methylation in 276 CpGs located in the promoters of 254 genes. Methylation changes were not present in circulating blood cells from type 2 diabetic patients. Exposure of islets from non-diabetic donors to high glucose for three days did not induce these methylation changes. An inverse correlation of gene expression and methylation was detected for some genes. Functional annotation of the differentially methylated genes pointed to pathways regulating beta cell dysfunction and death, and this was validated by RNA interference studies. Currently, second generation arrays are being used to interrogate an order of magnitude more CpGs. Taken together, these studies will help to unveil epigenetic disease mechanisms in islets in type 2 diabetes.

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K2

Riboflavin lowers blood pressure in hypertensive people with the *MTHFR* 677TT genotype

Helene McNulty*, JJ Strain, Mary Ward
Northern Ireland Centre for Food & Health, University of Ulster, Coleraine, BT52 1SA, UK
E-mail: h.mcnulty@ulster.ac.uk
Archives of Public Health 2014, **72(Suppl 1):**K2

Hypertension, defined as a systolic/diastolic blood pressure of 140/90 mmHg or greater, is estimated to carry a 3-fold increased risk of developing cardiovascular disease (CVD), while treating hypertension significantly reduces CVD events, and stroke in particular. Among the many risk factors involved, there is much recent interest in the role of genetic factors that might predispose to hypertension.

Evidence from genome-wide association studies has identified an association between blood pressure and the gene encoding the folate-metabolising enzyme, methylenetetrahydrofolate reductase (MTHFR), while recent meta-analyses of observational studies show an increased risk of hypertension in people homozygous for the 677C→T polymorphism in MTHFR. Riboflavin (vitamin B2) in the form of FAD acts as a cofactor for MTHFR and we have been studying its modulating role in relation to this polymorphism. The variant enzyme is known from molecular studies to become inactive as a result of having an increased propensity to dissociate from FAD, but our earlier work suggested that supplementation with low-dose riboflavin could stabilise MTHFR activity *in vivo* in homozygous individuals. In recent years we showed that CVD patients with the relevant *MTHFR* 677TT genotype (compared to CC or CT genotypes) had significantly higher blood pressure, and that blood pressure was highly responsive to riboflavin intervention, specifically in the TT genotype group [1]. Further investigations confirmed this gene-nutrient interaction in hypertensive patients (with and without overt CVD), and furthermore showed that the blood pressure lowering effect of riboflavin in the TT genotype group was independent of the number and type of antihypertensive drugs that they were taking [2].

Although the precise mechanism linking this polymorphism to hypertension remains to be established, it would appear that the biological perturbation that leads to higher blood pressure in individuals with the MTHFR 677TT genotype is modifiable by correcting the variant MTHFR enzyme through enhancing riboflavin status. Thus riboflavin, targeted specifically at this genetically at-risk group, may offer a personalized non-drug approach to managing hypertension.

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K3

The gut microbiome - a new target for understanding, diagnosing and treating disease

Jeroen Raes
Department Microbiology & Immunology KU Leuven, B-3000 Leuven, Belgium
E-mail: jeroen.raes@med.kuleuven.be
Archives of Public Health 2014, **72(Suppl 1):**K3

The functioning of the human body constitutes a complex interplay of human processes and 'services' rendered to us by the 1000 trillion microbial cells we carry. Disruption of this natural microbial flora is linked to infection, autoimmune diseases and cancer, but detailed knowledge about our microbial component remains scarce [1].

Recent technological advances such as metagenomics and next-generation sequencing permit the study of the various microbiota of the human body at a previously unseen scale. These advances have allowed the initiation of the International Human Microbiome Project, aiming at genomically characterizing the totality of human-associated microorganisms (the "microbiome") [2].

Here, I will present our work on characterizing the human intestinal flora based upon the analysis of high-throughput meta-omics (metagenomics, metatranscriptomics, metaproteomics) data. I will show how the healthy gut flora can be classified "enterotypes" that are independent from host nationality, age, BMI and gender, but linked to nutrition [3]. I will also show how metagenome-wide association studies (MGWAS) can lead to the detection of diagnostic markers for host properties and disease (e.g. in IBD, diabetes and obesity), and aid in further understanding on how the gut flora disturbances contribute to these pathologies. Finally, I will illustrate how gut microbiota-based treatment strategies are emerging, for example through Faecal Microbiota Transplantation (FMT).

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K4

Nutrigenetics and personalised/stratified approaches to the provision of dietary advice

Anne-Marie Minihane
Nutrition Department, Norwich Medical School, University of East Anglia (UEA), Norwich NR4 7TJ, UK
E-mail: A.Minihane@uea.ac.uk
Archives of Public Health 2014, **72(Suppl 1):**K4

Nutrigenetics refers to the interaction of genotype and diet composition to influence metabolism, health status and the risk of diet-related diseases. Gene variants influence food choice and appetite and therefore the intake of dietary components, their absorption, metabolism and tissue uptake along with their impact on molecular targets and physiological processes.

The first draft of the majority (~90%) of the sequence of the human genome was published in a *Nature* article entitled *'Initial sequencing and analysis of the human genome'* in 2001 [1] with the complete sequence (~99.7%) available in 2004 [2]. At the time such information was considered by many to be the panaceas and one of the greatest ever medical achievements, with genetic analysis likely to refine disease risk and prediction and the personalisation/stratification of interventions in order to afford maximum benefits.

Thirteen years on many consider progress based on the human genome to be limited, with the exception of the identification of the genetic basis of Mendelian (monogenic) disorders. Much of the estimated heritable component of disease risk and response to environmental change is unaccounted for. Based on available information, it appears that rather than being overestimated, the heritability is dark matter (i.e. it is real but we cannot see it yet), attributable to as yet undetected rare variants, or the underestimation of the impact of known variants. Genotype-diet-phenotype associations are not homogenous and influenced by a whole range of variables such as sex, ethnicity, drug use and other lifestyle variables which could in part account for the apparent 'hidden heritability' [3].

Specific examples of nutrigenetic interactions will be presented, in particular in the area of *APOE* genotype, along with some consideration of the practical, ethical, social and cost implications of the wider use of genetic information in public health and clinical practice.

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K5

Exploring future opportunities and barriers for business model concepts in personalized nutrition

Jo Goossens
shiftN, Naamsesteenweg 255, 3001 Heverlee, Belgium
E-mail: jg@shiftn.com
Archives of Public Health 2014, **72(Suppl 1):**K5

Facing a growing health issue as a result if inappropriate dietary patterns, our society seems unable to convince individuals to adjust nutrition and lifestyle in an appropriate and lasting way to reduce health risks (and costs).

The sequencing of the human genome, heralded great expectations for a revolution in health care with the road to personalised health care and its extension to personalised nutrition. Although the earliest attempts fauiled to commercialise this approach, personalised nutrition, where nutrigenomics, dietary intake data, biomarker analyses and other general life-style information can be combined hold still great promises to make nutritional and dietary advice more individually acceptable and thus more effective.

Food4Me, an EU FP7 project, explores all aspects of personalized nutrition, from consumer response, scientific basis, technological tools to legal and ethical issues and value creation models. The aim is to understand what is possible, for whmo this approach could be interesting and how such an offering could succeed in the future in society.

Research based on current market offerings in this field, interviews with a wide range of stakeholders and consumer reactions to personalised nutrition concepts, shows that the key to personalised nutrition is NOT about improving nutritional advise or communicating more and better about it, it is about coaching people to bring about a lasting behavior change. THIS has more to do with behavioral science than nutrition and biology. From these analyses we built a personalised nutrition system. Dietary behaviour change sits in the center of the system and is influenced by 8 key driving forces: effectiveness of nutritional advice, economic feedback signals, force of dietary habits, psychological ambivalence (about diet and health), acceptance of genetic diagnostic information and the reliability of the risk/need profile assessment. Of course scientific excellence, especially in terms of evidence of genotype and biomarker relationships with nutrient intake, still needs to be further developed but it will to no use if the advice is not provided through good quality nutrition and lifestyle coaching or if there is no willingness to accept this. The latter will obviously require stories of positive experiences or otherwise some form of coercion.

The opportunity for novel business model concepts was then explored in 4 future scenarios about the evolution of the concept of health and the logic of health care systems in Europe.

ORAL PRESENTATIONS

O1

Children's stress influences their diet, physical activity and adiposity: longitudinal behavioural and hormonal pathways

Nathalie Michels[1*], Isabelle Sioen[1], Liesbet Boone[2], Caroline Braet[2], Els Clays[1], Inge Huybrechts[1], Barbara Vanaelst[1], Stefaan De Henauw[1]
[1]Department of Public Health, Ghent University, Ghent, Belgium; [2]Department of Developmental, Personality and Social Psychology, Ghent University, Ghent Belgium
Archives of Public Health 2014, **72(Suppl 1):**O1

Background: Psychosocial stress and adiposity are important public health threats that have been associated with each other. Longitudinal studies are needed to reveal the directionality and underlying behavioural and hormonal factors. In young children, literature is scarce. We aim to study the longitudinal associations of children's stress with lifestyle and adiposity.

Materials and methods: In 312 Belgian children (5-12y) of the ChiBS study, the two-year longitudinal stress-lifestyle-adiposity relation was examined. Stress data (sum score of negative events, problem behaviour and negative emotions), lifestyle (food consumption, psychological eating behaviour, physical activity by accelerometry, screen time, sleep duration) and adiposity (BMI, fat% by BodPod, waist) were measured. At baseline, also salivary cortisol levels (4 samples, 2 days) were determined as a biomarker for stress. Multilevel time modelling examined the cross-sectional relation of cortisol with diet. Mplus cross-lagged analyses tested the longitudinal stress-lifestyle-adiposity relations and moderation.

Results: Children with a high stress score reported more sweet food consumption, psychological eating behaviour (emotional eating, external eating, restrained eating) and physical activity. Some of these relations were sex- and age-dependent. No effects on sleep duration were found. The stress effect on adiposity was depending on moderators. Sweet food consumption and cortisol were enhancing moderators: stress increased adiposity in children with high sweet food consumption (BMI, $p=0.020$) or high cortisol awakening response (waist, $p=0.030$). Physical activity was a protective moderator: stress decreased adiposity in children with high physical activity (fat%, $p=0.025$). In the other direction, high BMI ($p<0.001$), high fat% ($p<0.001$) and psychological eating behaviour ($p=0.014$) also increased stress. High cortisol (overall levels, awakening response and diurnal slope) was associated with an unhealthy diet especially with the sweet foods.

Conclusions: The association of cortisol with diet supports the theory of cortisol–induced comfort food preference. Indeed, children's stress deteriorates their diet which stimulates adiposity. On the other hand, stress can also enhance physical activity which inhibits adiposity. This creates a perspective for multi-factorial obesity prevention, targeting stress and lifestyle factors in parallel. Concerning lifestyle, the environment should be an 'activity encouraging, healthy food zone' that minimizes opportunities for stress-induced eating. Concerning stress, appropriate stress coping skills should be acquired. Moreover, psychological support for obese children should be organized.

O3

Genetic and phenotypic determinants of flavonoid absorption and metabolism: the COB study

Sumanto Haldar*, Noemí T Hernandez, Luisa Ostertag, Peter Curtis, Aedín Cassidy, Anne Marie Minihane
Department of Nutrition, Norwich Medical School, University of East Anglia, Norwich, NR4 7TJ, UK
E-mail: s.haldar@uea.ac.uk
Archives of Public Health 2014, **72(Suppl 1):**O3

Background: Flavonoids are present in numerous plant foods, including fruits, vegetables, teas, red wine, cocoa, herbs, and spices. Epidemiological data, along with evidence from cell, animal, and limited human intervention trial studies suggest that flavonoids can improve cardiovascular health and reduce the risk of other ageing-related diseases [1-6]. However, the absorption and metabolism of flavonoids, their metabolite profile, and their associated health benefits are highly heterogeneous [7], with the aetiological basis of this variability currently unknown. Our recent stable isotope study, which fed an oral bolus dose of 500 mg ^{13}C-labeled cyanidin-3-glucoside to healthy men showed a wide inter-individual variability in anthocyanin metabolism [7].
Methods: The COB (Chocolate, Orange and Blackberry) study is examining the influence of genotype, age, gender and composition of the intestinal microbiota on the absorption, metabolism and elimination (AME) of flavonoids. It is an acute feeding study with mixed-flavonoids (containing flavan-3-ols, flavanones and anthocyanins) conducted in 120 men and 120 women (Caucasians, of European Origin), from two different age groups, 18-30 y and 65-77 y. The parent flavonoids and their metabolites will be measured in plasma and urine for up to 48 hours post-feeding. Participants' genotypes will be established using exome sequencing and targeted genotyping for Single Nucleotide Popymorphisms (SNPs) in genes associated with i) pathways involved in flavonoid metabolism (in particular phase 1 and 2 enzymes); ii) genes that may alter the intestinal microbial composition; and iii) those modulating gut physiology. The composition of the gut microflora will be analysed by pyrosequencing of 16S rRNA and metagenomic technologies.
Results: The COB intervention is currently ongoing and due for completion in October 2014. 142 tagging SNPs have been identified in candidate genes involved in flavonoid absorption, metabolism and elimination including LPHs (deglycosylation), UGTs (glucuronidation), SULTs (sulfation), COMT (methylation) and ABC transporters (such as MRP2) which will be analysed in our study population. In order to generate pilot data to help inform the genotyping approach in the COB study, the impact of a selection of these SNPs (Table 1) on flavonoid metabolism and clinical endpoints is being determined in a completed one year trial involving 47 post-menopausal women [1].
Implications: The proposed work will advance current knowledge regarding the genetic and physiological determinants of flavonoid absorption and metabolism. Such information would allow greater refinement of current recommended intakes of flavonoid rich foods, such as fruits and vegetables
Trial registration: ClinicalTrials.gov identifier: NCT01922869, ISRCTN14271372

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O4

Alternatives for nitrate and nitrite in fermented meat products: potential contribution of the nitric oxide synthase activity of coagulase-negative staphylococci

María Sánchez Mainar*, Stefan Weckx, Luc De Vuyst, Frédéric Leroy
Research Group of Industrial Microbiology and Food Biotechnology, Faculty of Sciences and Bioengineering Sciences, Vrije Universiteit Brussel, B-1050 Brussels, Belgium
E-mail: Maria.Sanchez@vub.ac.be
Archives of Public Health 2014, **72(Suppl 1):**O4

Background: Nitrosomyoglobin, which is the cured colour of fermented meat products, results from the interaction between muscle-based myoglobin and nitric oxide (NO) [1]. NO originates from the addition of nitrate and/or nitrite as curing agents to the meat batter. During fermentation, nitrate is reduced into NO-yielding nitrite by coagulase-negative staphylococci (CNS), present in the meat or added as starter culture [2]. However, health concerns related to the consumption of cured meats are leading to research for alternatives to generate the cured colour. A yet poorly explored pathway could potentially be based on the action of nitric oxide synthase (NOS), which produces NO from arginine. Bacterial NOS activity has only been scantily described, particularly its potential presence in meat-related bacteria and its dependency on environmental conditions. Based on preliminary attempts [3], and because up to now none of the sequenced *Lactobacillus* species contain a NOS homologue [4], this study focused on meat-related CNS.
Materials and methods: A genotypic screening for the presence of the NOS-encoding gene and a phenotypic screening for the conversion of arginine via NOS activity and other alternative pathways metabolising arginine were performed for 88 CNS strains. Also a complementary screening for potential NOS-stimulating conditions and a kinetic analysis of possible NOS activities in CNS were done in laboratory fermentors and meat models.
Results: The genetic potential for NOS activity was frequently found among CNS strains. The phenotypic screening confirmed that arginine

Table 1(abstract O3)

SNP (Gene)	Variant 1	number (%)	Variant 2	number (%)	Variant 3	number (%)
rs4988235 (LPH)	AA	19 (40%)	AG	21 (45%)	GG	6 (13%)
rs3760091 (SULT)	CC	10 (21%)	GC	19 (40%)	GG	18 (38%)
rs4788068 (SULT)	AA	8 (17%)	GA	21 (45%)	GG	18 (38%)
rs2273697 (MRP2)	AA	5 (11%)	AG	20 (43%)	GG	22 (47%)
rs737865 (COMT)	AA	25 (53%)	AG	21 (45%)	GG	1 (2%)
rs4680 (COMT)	AA	13 (28%)	AG	23 (49%)	GG	11 (23%)

metabolism was common, which resulted in mixtures of citrulline and mostly ornithine, with considerable variability on species and strain level, indicative of arginine deiminase activity. The production of citrulline without ornithine formation, indicative of potential NOS activity, was not found under the conditions tested, except for the strain *S. haemolyticus* G110. However, kinetic experiments indicated that *S. haemolyticus* G110 was not able to demonstrate NO-driven colour formation in fermented meats, highlighting the importance of technological adaptation of functional candidate strains. Attempts to express the *nos* gene in other CNS strains were unsuccessful, suggesting that the genetic potential is not commonly expressed by CNS.

Conclusions: The use of NOS-positive bacterial cultures for nitrate and nitrite cutback in fermented meats is not straightforward. A bottleneck seems to be on the gene expression level, whereas phenotypically positive strains also need to be technologically adapted to the meat fermentation process.

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O5

Prebiotics supplementation improves the endothelial dysfunction in n-3 PUFA-depleted ApoE$^{-/-}$ mice

Emilie Catry[1], Géraldine Rath[2], Barbara D Pachikian[1], Audrey M Neyrinck[1], Patrice D Cani[1], Chantal Dessy[2], Nathalie M Delzenne[1*]
[1]Metabolism and Nutrition Research Group, Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium; [2]Pole of Pharmacology and Therapeutics, Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, Brussels, Belgium
E-mail: nathalie.delzenne@uclouvain.be
Archives of Public Health 2014, **72(Suppl 1):**O5

Background: Our previous studies demonstrated that dietary n-3 polyunsaturated fatty acids (PUFA) deficiency promotes the development of non-alcoholic fatty liver disease in mice, and that modification of gut microbiota composition by prebiotics (non-digestible fructans) can improve the hepatic steatosis and serum lipids in this model [1,2]. The present study has been designed to analyze the potential involvement of prebiotic supplementation on endothelial dysfunction in n-3 PUFA-depleted ApoE knock-out mice.

Material and methods: Wild-type (WT, n=6) and ApoE$^{-/-}$ (KO, n=6) mice were fed with a n-3 PUFA-depleted diet for 12 weeks. Fifteen days before the end, WT (n=3) and KO (n=3) mice were supplemented with fructans as prebiotics (PRE). Second and third generation mesenteric arteries were isolated and mounted on a wire myograph. After normalization, arteries were contracted with a KCl-enriched (50mM) solution. The endothelial-dependent relaxation was evaluated after addition of increasing doses of acetylcholine.

Results: The analysis of morphological parameters showed that mesenteric micro-arteries isolated from n-3 PUFA depleted-KO mice supplemented with PRE (KO-DEF-PRE) present an significant increasing by 20% in mean diameter and develop also an significant increasing by 35% in the basal tone compared to vessels from other groups (KO-DEF or WT-DEF). Similarly, KO-DEF-PRE micro-arteries contracted significantly more to KCl-enriched solution than vessels isolated from other groups. Finally, we measured the relaxation evoked by acetylcholine: KO-DEF-PRE micro-arteries relaxed significantly more compared to KO-DEF mice isolated micro-arteries (61.27±0.343 %KCl max vs 80.513±2.542 %KCl max, $p<0.01$). This effect was blunted in the presence of COX inhibitor, indomethacin.

Conclusion: Our results suggest that fifteen days of prebiotic supplementation is sufficient to alter morphological and contractile parameters in the mesenteric bed. Importantly, prebiotic supplementation is also able to prevent the endothelial dysfunction observed in KO-DEF mice, independently of the contractile modifications. Results obtained in the presence of indomethacin appoint prostanoids as possible molecular targets, in addition to the NO/NOS pathway. Further analyses are now performed to relate changes in gut functions to cardiovascular alterations.

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O6

Inulin-type fructan degradation capacity of interesting butyrate-producing colon bacteria and cross-feeding interactions of *Faecalibacterium prausnitzii* DSM 17677^T with bifidobacteria

Frédéric Moens*, Audrey Rivière, Marija Selak, Luc De Vuyst
Research Group of Industrial Microbiology and Food Biotechnology (IMDO), Faculty of Sciences and Bio-engineering Sciences, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium
E-mail: fmoens@vub.ac.be
Archives of Public Health 2014, **72(Suppl 1):**O6

Background: Inulin-type fructans have already been studied with respect to their stimulation of bifidobacteria and butyrate-producing colon bacteria, such as *Anaerostipes caccae* and *Roseburia* spp. However, much less is known about their effects on other butyrate-producing colon bacteria, such as *Butyricicoccus pullicaecorum*, *Eubacterium* spp. and *Faecalibacterium prausnitzii* and the interactions of these species with bifidobacteria. This study aimed at investigating the kinetics of inulin-type fructan degradation and organic acid and gas production by *B. pullicaecorum* DSM 23266^T, *E. hallii* L2-7, *E. rectale* CIP 105953^T, and *F. prausnitzii* DSM 17677^T and the possible interactions between *F. prausnitzii* DSM 17677^T and bifidobacteria.

Materials and methods: All butyrate-producing strains were studied during screening experiments (100-ml scale) and monoculture fermentations (1.5-l scale) in a medium for colon bacteria (MCB) containing either fructose, oligofructose, or long-chain inulin as an energy source, supplemented with acetate. Coculture fermentation experiments (1.5-l scale) in MCB were performed with *F. prausnitzii* DSM 17677^T and *Bifidobacterium breve* Yakult, *Bifidobacterium adolescentis* LMG 10734, *Bifidobacterium angulatum* LMG 11039^T (oligofructose), and *Bifidobacterium longum* LMG 11047 (oligofructose or inulin as a substrate).

Results: *Butyricicoccus pullicaecorum* DSM 23266^T and *E. hallii* L2-7 degraded fructose only, resulting in the production of butyrate, H_2 and CO_2. *Eubacterium rectale* CIP 105953^T produced lactate and butyrate as well as H_2 and CO_2 out of fructose and inulin-type fructans. *Faecalibacterium prausnitzii* DSM 17677^T produced butyrate, formate, and traces of lactate, together with CO_2 out of fructose, oligofructose, and inulin. Both oligofructose-consuming, butyrate-producing strains degraded all oligofructose fractions simultaneously, indicating an extracellular degradation mechanism. During coculture fermentation experiments, oligofructose (by all bifidobacteria, except for *B. breve* Yakult) and inulin (by *B. longum* LMG 11047) were converted into acetate, lactate, and formate. *Faecalibacterium prausnitzii* DSM 17677^T was cross-fed on this acetate resulting in the production of its metabolites. However, only low amounts of butyrate were produced during the coculture fermentations with *B. angulatum* LMG 11039^T and *B. longum* LMG 11047, since *F. prausnitzii* DSM 17677^T did not manage to compete well for the oligofructose substrate in the presence of *B. angulatum* LMG 11039^T and *B. longum* LMG 11047, and for the inulin substrate in the presence of *B. longum* LMG 11047.

Conclusion: Besides cross-feeding interactions between bifidobacteria and butyrate-producing colon bacteria, competition for the available inulin-type fructans between these colon bacteria may occur. As a result, fast degraders such as bifidobacteria are favoured compared to acetate-depending butyrate producers.

O7

Role of ciliary dysfunction in a new model of obesity and non-alcoholic steatohepatitis: the *foz/foz* mice

Laurence Poekes[1*], Vanessa Legry[1], Geoffrey Farrell[2], Isabelle Leclercq[1]
[1]Laboratory of Hepato-Gastroenterology, UCL, Brussels, Belgium; [2]ANU, Canberra, Australia
E-mail: laurence.poekes@uclouvain.be
Archives of Public Health 2014, **72(Suppl 1):**O7

Introduction: *Foz/foz* mice are deficient for Alms1, a ubiquitous protein essential for proper primary cilium function. They are prone to insulin resistance, obesity and diabetes, a phenotype accelerated by high-fat diet (HFD) feeding. Their unique metabolic phenotype has been linked to hyperphagia resulting from abnormal ciliary function in the central nervous system [1]. The aim of our study is to verify the dependence of the phenotype on over-feeding and to explore the role of Alms1 deficiency in intestinal energy absorption.
Materials and methods: Male *foz/foz* (Alms1-/-) and wild-type (WT) littermates were fed a HFD for 4 weeks to evaluate their food intake, metabolic parameters (glucose tolerance, steatosis, adiposity) and tissue inflammation. We next performed a pair-feeding experiment in which *foz/foz* mice had access to the exact same amount of HFD consumed by WT the day before. Lipid absorption was evaluated by oral fat tolerance test and total lipid content in the feces.
Results: As expected, *foz/foz* mice ate more (18.2 vs 14.1 kcal/d, p<0.001), became more obese (42.5 vs 26.8g, p=0.01) and glucose intolerant than WT mice fed a HFD (p=0.008). Unlike WT mice, they also developed steatosis, adipose tissue and liver inflammation. In the pair-feeding experiment, *foz/foz* mice and WT mice were fed iso-calorically. However, *foz/foz* mice gained more weight (+54.3% vs +29.7%, p<0.001), were more glucose intolerant and presented higher adipose inflammation than WT mice. To explain the metabolic alterations, we hypothesized that Alms1 deficiency in intestine could contribute to increased nutrient absorption. We found that fecal lipid content was lower in *foz/foz* than in WT mice matched for HFD intake (31.3 vs 45.9 mg/24h feces, p=0.01). Moreover, upon oral fat load, *foz/foz* mice had higher plasma triglyceride levels than WT mice (79.1 vs 15.7 mg/dL, p< 0.05).
Conclusion: These results suggest that, beside causing hyperphagia, Alms1 deficiency increased dietary energy extraction, that could participate to the metabolic phenotype leading to insulin resistance and obesity. The understanding of the mechanisms at play may uncover new potential therapeutic targets.

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O8

Foodborne cereulide causes beta cell dysfunction and apoptosis

Roman Vangoitsenhoven[1*], Dieter Rondas[1], Inne Crèvecoeur[1], Wannes D'Hertog[1], Matilde Masini[2], Mirjana Andjelkovic[3], Joris Van Loco[3], Christophe Matthys[1], Chantal Mathieu[1], Lut Overbergh[1], Bart Van der Schueren[1]
[1]Clinical and Experimental Medicine and Endocrinology, KU Leuven, Leuven, Belgium; [2]Department of Translational Research and of New Surgical and Medical Technologies, University of Pisa, Italy; [3]Food, Medicines, and Consumer Safety, Scientific Institute of Public Health, Brussels, Belgium
E-mail: Roman.Vangoitsenhoven@med.kuleuven.be
Archives of Public Health 2014, **72(Suppl 1):**O8

Background: Environmental factors play a major role in the rising prevalence of type 1 and type 2 diabetes mellitus. Cereulide is a lipophilic peptide that is often found at low concentrations in starchy food. It is a culprit to consider in this era of prepackaged meals.
Materials and methods: Mouse and rat insulin producing beta cell lines, MIN6 and INS-1E respectively, as well as whole mouse islets, isolated from 2 week old C57Bl/6J mice, were exposed to cereulide concentrations ranging from 0.05ng/ml to 5ng/ml for 24 and 72h. Cell death was evaluated by a Hoechst/Propidium Iodide assay, and compared to cell death in human hepatocellular HepG2 and monkey fibroblast-like COS-1 cells. Subsequently, MIN6 cells were exposed to low concentrations of cereulide (0.15 - 0.5 ng/ml) for 24h and glucose-stimulated insulin secretion was evaluated as well as mechanisms of toxicity by mRNA profiling, electron microscopy and caspase activation and cytochrome c release assay.
Results: Cereulide exposure caused cell death in MIN6, INS-1E and pancreatic islets, but not in HepG2 or COS-1E cells (Table 1). Caspase 3/7 activation confirmed the apoptotic cell death process. Glucose-stimulated insulin secretion decreased from 10.48 ± 3.33 fold to 2.01 ± 0.51 ($P < 0.05$) in MIN6 cells after 24h exposure with 0.25 ng/ml cereulide. Exposure to 0.25ng/ml cereulide induced markers of mitochondrial stress, including PUMA (p53 upregulated modulator of apoptosis; 271 ± 77 % of control; $P < 0.05$) but also markers of ER stress, such as CHOP (CCAAT/-enhancer-binding protein homologous protein; 641 ± 190 % of control; $P < 0.01$). EM revealed swelling and loss of mitochondria, and cytoplasmic cytochrome c release confirmed mitochondrial cell death signalling (360 ± 83 % of control after exposure to 0.5 ng/ml for 24h ($P < 0.05$).
Conclusion: Cereulide, a toxin frequently found in prepackaged or prepared starchy meals, increases levels of mitochondrial and ER stress markers in beta cells of rats and mice, even at low doses. In a dose dependent way, it also leads to impaired beta cell function and apoptosis. Cereulide might thus be involved in the current diabetes.

POSTER PRESENTATIONS

P1

Water kefir as a promising low-sugar probiotic fermented beverage

David Laureys, Luc De Vuyst*
Research Group of Industrial Microbiology and Food Biotechnology, Faculty of Sciences and Bioengineering Sciences, Vrije Universiteit Brussel, B-1050 Brussels, Belgium
E-mail: ldvuyst@vub.ac.be
Archives of Public Health 2014, **72(Suppl 1):**P1

Background: Water kefir is a slightly sweet, acidic, fruity, sparkling, and slightly alcoholic fermented beverage produced with water kefir grains, the latter consisting of polysaccharides and micro-organisms. The micro-organisms involved in water kefir fermentation comprise yeast, lactic acid bacteria, bifidobacteria, and acetic acid bacteria. Some strains of micro-organisms in water kefir might possess probiotic activity. Also, water kefir is a beverage with relatively low sugar content, providing an interesting alternative to sugary soft drinks. To be able to exploit water kefir for its probiotic potential or as a low-sugar soft-drink, in-depth research is needed to unravel the species diversity and community dynamics of water kefir fermentation. In particular, substrate consumption and metabolite production in water kefir have not been studied until now.

Table 1(abstract O8) Apoptosis induced after 24h exposure to cereulide (mean percentage ± SEM).

	MIN6 (n=5)	INS-1E (n=4)	HepG2 (n=3)	COS (n=3)	Islets (n=3)
Medium	7.3 ± 1.3	2.5 ± 0.3	5.8 ± 0.6	1.2 ± 0.6	3.1 ± 1.2
0.05 ng/ml cereulide	5.9 ± 1.0	3.2 ± 0.5	6.6 ± 2.1	1.6 ± 0.4	3.9 ± 1.5
0.25 ng/ml cereulide	31.6 ± 5.8 *	58.1 ± 11.4 *	6.9 ± 1.5	2.9 ± 0.7	8.6 ± 2.4
0.5 ng/ml cereulide	43.6 ± 6.1 *	100.0 ± 0.0 *	11.9 ± 2.5	2.6 ± 0.6	49.2 ± 9.0
5 ng/ml cereulide	100.0 ± 0.0 *	100.0 ± 0.0 *	7.7 ± 2.3	4.3 ± 0.9	96.4 ± 3.5*

* $p \leq 0.05$ vs control

Materials and methods: Water kefir fermentation processes were followed as a function of time for 192 h. At each sampling point, the pH, microbial counts (lactic acid bacteria, yeast, and acetic acid bacteria), and residual substrate and metabolite concentrations were measured, including volatile aroma compounds. The species diversity was unravelled through both culture-dependent and culture-independent techniques.
Results: Species diversity analyses indicated that the most important microbial species were *Lactobacillus casei/paracasei*, *Lactobacillus harbinensis*, *Lactobacillus hilgardii*, *Bifidobacterium psychraerophilum/crudilactis*, *Saccharomyces cerevisiae*, and *Dekkera bruxellensis*. This microbial species diversity was similar in the water kefir liquor and on the water kefir grains, and remained stable during the whole fermentation process. Some strains of these species, such as *Lb. casei* and *Bifidobacterium* spp., might possess probiotic activities. Sucrose, the major substrate of the fermentation was completely converted after 24 h of fermentation, which coincided with the production of the water kefir grain polysaccharide. The main metabolites of the fermentation were ethanol and lactic acid, whereas glycerol, acetic acid, and mannitol were produced in low concentrations. The most prevailing volatile aroma compounds (relative to their threshold values) were ethyl acetate, isoamyl acetate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate. These are fruity esters which might have a positive impact on the aroma of the end-product.
Conclusions: In this study the species diversity, community dynamics, substrate consumption, and metabolite production during water kefir fermentation were described in detail. This work provides a basis for further developments of water kefir as healthy, low-sugar, probiotic fermented beverage.

P3

A pilot study on the impact of maternal diet and preconception body mass index on breast milk macronutrient composition

Goele Jans[1*], Rivka Turcksin[2], Bart Van der Schueren[3,4], Christophe Matthys[3,4], Roland Devlieger[1,2,3,4,5]
[1]Department of Development and Regeneration, KU Leuven, Leuven, 3000, Belgium; [2]Faculty of Medicine, Nursing and Midwifery, KU Leuven, Leuven, 3000, Belgium; [3]Department of Clinical and Experimental Medicine, KU Leuven, Leuven, 3000, Belgium; [4]Department of Endocrinology, UZ Leuven, Leuven, 3000, Belgium; [5]Department of Obstetrics and Gynecology, UZ Leuven, Leuven, 3000, Belgium
E-mail: goele.jans@med.kuleuven.be
Archives of Public Health 2014, **72(Suppl 1):**P3

Background: Breastfeeding is the number one recommendation to serve newborns and young children with all nutrients they need for a healthy growth and development. The aim of this pilot study was to investigate the impact of maternal diet and preconception Body Mass Index (BMI) on the macronutrient composition of breast milk.
Materials and methods: A cross-sectional study was conducted at the maternity units of the University Hospital of Leuven, Belgium. Postpartum women, who delivered a term (≥37 weeks of gestation), did not smoke, were normoglycaemic and who were not on predefined medication, were invited to participate. Participants completed a 24 dietary recall and donated a breast milk sample of 1.5-2cl at day 4 post-delivery. The samples were collected during the first feeding in the morning by use of a vacuum pump. Each sample was analyzed for macronutrients (carbohydrates, protein, fats) and energy with the MIRIS® Human Milk Analyzer. The pre-pregnancy weight and length were assessed from the online medical patient file to calculate the pre-pregnancy BMI. Further baseline characteristics included maternal age, ethnicity and parity. Pregnancy outcomes included delivery mode, the use of combined spinal epidural, gestational age, birth weight and gender of the baby.
Results: Analyses have been performed on samples from 33 postpartum women. There were no differences in baseline characteristics. A positive correlation was found between maternal BMI and carbohydrate concentration in breast milk (r=0.3778; p=0.030). A significant difference in human milk carbohydrate concentration was seen when dividing into groups, indicating a higher concentration in obese women (BMI ≥30 kg/m^2) compared to normal weight women (BMI 18.5-24.9 kg/m^2) (7.3±0.9 g/100ml versus 6.6±0.6 g/100ml; p=0.017). A linear regression showed that every increase of one unit of BMI presented an increase of 0.045g in mean carbohydrate concentration. No correlation was found between the maternal diet and the concentration of macronutrients and energy in breast milk.
Conclusions: Breast milk composition partially depends from maternal BMI, with only a higher carbohydrate concentration in the obese women. The maternal diet seems to have no impact on the macronutrient milk composition. This is not consistent with results of other studies, reporting an impact of maternal fat consumption on the fat composition of breast milk. The small study group does not allow drawing strong conclusions. Analyses with a larger study group are needed.

P4

Do Roux-en-Y gastric bypass patients meet the dietary guidelines?

Ina Gesquiere[1*], Kelly Van Meerbeeck[2], Veerle Foulon[1], Patrick Augustijns[1], Matthias Lannoo[3], Ann Meulemans[4], Bart Van der Schueren[4], Christophe Matthys[4]
[1]Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Leuven, Belgium; [2]Department of Health and Technology, Leuven University College, Leuven, Belgium; [3]Department of Pathology Abdominal Surgery, University Hospitals Leuven, Leuven, Belgium; [4]Clinical and Experimental Endocrinology, KU Leuven/University Hospitals Leuven, Leuven, Belgium
E-mail: ina.gesquiere@pharm.kuleuven.be
Archives of Public Health 2014, **72(Suppl 1):**P4

Background: The prevalence of obesity has increased to epidemic proportions and, as a result, the number of bariatric surgeries has increased worldwide. To date, bariatric surgery remains the sole medical intervention that achieves considerable and sustained weight loss. As both obesity and bariatric surgery are associated with nutritional deficiencies, the aim of this study was to evaluate the dietary intake of macro- and micronutrients in patients before and after Roux-en-Y gastric bypass (RYGB).
Methods: A prospective observational study was performed at University Hospitals Leuven, Belgium. Patients completed a dietary record of two non-consecutive days before RYGB and 1 and 3 months after RYGB. Intake of macronutrients and micronutrients was calculated for the different time-points. Paired sample t-tests were performed to analyse differences between time-points.
Results: Conclusions: The intake of macro- and micronutrients is markedly decreased one month after RYGB. At three months post-surgery, the intake of macronutrient increases, but the micronutrient intake remains identical at a worryingly low level. Our data clearly suggest that nutritional guidance is essential following bariatric surgery.

Table 1(abstract P4) Intake of macronutrients at different time-points, shown as mean±SD.

n=22	Intake pre-RYGB	Intake 1 month post-RYGB	Intake 3 months post-RYGB	Significance
Carbohydrates (g)	245.2±72.4	81.8±39.1	110.9±51.42	1,2
Proteins (g)	87.3±23.8	37.2±16.6	48.0±14.4	1,2,3
Fat (g)	92.2±40.4	20.5±12.6	36.3±16.2	1,2,3

1 p<0.01:pre-op vs post-op 1 month; 2 p<0.01:pre-op vs post-op 3 months; 3 p<0.01:post-op 1 month vs post-op 3 months

Table 2(abstract P4) Intake of micronutrients at different time-points, shown as mean±SD.

	Intake pre-RYGB (32 patients)	Intake 1 month post-RYGB (28 patients)	Intake 3 months post-RYGB (26 patients)	Significance
Ca (mg)	970.4±519.6	638.4±287.9	695.1±352.3	
Fe (mg)	12.6±3.7	5±2.9	6.0±1.8	1,2
Cu (mg)	2.1±1.5	1.0±0.9	4.9±18.6	
Zn (mg)	46.6±92.1	10.2±21.1	6.6±3.7	
Vitamin A (μg)	962.8±405.2	721.5±490.0	787.5±716.6	
Vitamin B1 (mg)	1.7±0.7	0.6±0.3	0.8±0.3	1,2
Vitamin B12 (μg)	5.4±2.5	2.3±1.5	3.3±1.8	1,2
Vitamin C (mg)	138.9±83.8	70.3±56.7	85.1±52.2	1,2
Vitamin D (μg)	8.4±5.1	5.2±3.3	4.2±3.2	

1 p<0.01:pre-op vs post-op 1 month; 2 p<0.01:pre-op vs post-op 3 months; 3 p<0.01:post-op 1 month vs post-op 3 months

Trial registration: Clinicaltrials.gov #NCT01571180.
Acknowledgements: I.G. receives a PhD scholarship from the Agency for Innovation by Science and Technology, Flanders.

P5

Influence of birth weight on calcaneal bone stiffness in Belgian pre-adolescent children

Karen Van den Bussche[1*], Nathalie Michels[1], Luis Gracia-Marco[2,3], Diana Herrmann[4], Gabriele Eiben[5], Stefaan De Henauw[1,6], Isabelle Sioen[1,7]
[1]Department of Public Health, Ghent University, 9000 Ghent, Belgium; [2]GENUD (Growth, Exercise, NUtrition and Development) Research Group, University of Zaragoza, Zaragoza, Spain; [3]School of Sport and Health Sciences, University of Exeter, Exeter, UK; [4]BIPS - Institute for Epidemiology and Prevention Research GmbH, Bremen, Germany; [5]Department of Pediatrics, Institute of Clinical Sciences, The Queen Silvia Children's Hospital, Sahlgrenska Academy at University of Gothenburg, Göteborg, Sweden; [6]Department of Health Sciences, Vesalius, University College Ghent, Ghent, Belgium; [7]FWO, Research Foundation Flanders, Egmontstraat 5, 1000 Brussels, Belgium
E-mail: Karen.VandenBussche@ugent.be
Archives of Public Health 2014, **72(Suppl 1):**P5

Background: Several studies have shown associations between birth weight and adult bone mass. However, it is uncertain whether that influence of birth weight is already visible in childhood. This study investigated the relation between birth weight and calcaneal bone stiffness in a large sample of Belgian healthy pre-adolescent children.
Materials and methods: Participants were 827 children (3.6–11.2 y, 51.6% boys) from the Belgian cohort of the IDEFICS study. Birth weight was obtained using a parental questionnaire and quantitative ultrasound (QUS) measurements were performed to determine the calcaneal Broadband Ultrasound Attenuation (BUA), Speed of Sound (SOS) and Stiffness Index (SI) using Lunar Achilles Device.
Results: The average birth weight was 3435.7 ± 512.0 g for boys and 3256.9 ± 471.1 g for girls. The average calcaneal QUS measurements were equal to 89.6 ± 24.0 (23.3 to 153.9) dB/MHz for BUA, 1621.4 ± 49.6 (1516.3 to 1776.5) m/sec for SOS and 92.8 ± 15.6 (49.0 to 163.0) for SI. Birth weight was positively associated with BUA ($r = 0.13$; $p = 0.002$) and with SOS ($r = -0.16$; $p < 0.001$). The associations remained after correcting for age and sex in multiple regression analyses, but disappeared after correcting for anthropometric covariates.
Conclusions: Our findings suggest that birth weight, as a rough proxy indicator for genetic and environmental influences during intrauterine life, is associated with BUA and SOS in pre-adolescent children and may therefore influence the risk of osteoporosis later in life. Further studies using QUS are needed to investigate the consistency of the results of this study.
Acknowledgements: On behalf of the IDEFICS consortium.

P6

Supplementation with crude rhubarb extract lessens liver inflammation and hepatic lipid accumulation in a model of acute alcohol-induced steato-hepatitis

Audrey M Neyrinck[*†], Usune Etxeberria[†], Alexandra Jouret, Nathalie M Delzenne
Louvain Drug Research Institute, Metabolism and Nutrition Research Group, Université catholique de Louvain, Brussels, Belgium
E-mail: audrey.neyrinck@uclouvain.be
Archives of Public Health 2014, **72(Suppl 1):**P6

Background: Binge consumption of alcohol is an alarming global health problem and is implicated in the pathophysiology of alcoholic liver disease (ALD) [1]. In its early stages, ALD it is characterized by fatty liver [1]. Moreover, numerous studies are supporting the concept that beyond this hepatic steatosis physiological processes, inflammation occurred in the progression of the disease [2]. Strategies directed to reduce fat accumulation and local hepatic inflammation caused by alcohol consumption might succeed blocking the evolution of ALD. In this sense, natural plants rich in bioactive constituents are attracting a growing interest as new therapeutic agents. Several studies have reported health benefits coming from rhubarb extract rich in anthraquinones [3,4]. The aim of the present study was to test the potential hepatoprotective effects of rhubarb extract supplementation in a model of acute alcohol-induced steato-hepatitis.
Materials and methods: Male C57Bl6J mice were fed with a control diet supplemented or not with 0.3% rhubarb extract (Laboratoires Ortis, Belgium). After 17 days, mice received a huge dose of ethanol (6g/kg bw) and were sacrificed 6 hours after the alcohol challenge. Liver oil red O staining and hepatic lipid contents were determined to assess hepatic steatosis whereas the expression of pro-inflammatory markers were analysed in the liver tissue by quantitative PCR to assess hepatic inflammation.
Results: Ethanol administration caused a massive increase of hepatic triglycerides and, in a lesser extent, in total cholesterol inside the liver tissue (table 1). Rhubarb extract decreased hepatic triglyceride content. This effect was confirmed by histological analysis (figure 1). Interestingly, rhubarb extract supplementation blunted the ethanol-induced F4/80 expression, suggesting a lower recruitment of inflammatory cells inside the liver (figure 2). Moreover, proinflammatory markers such as TNF-α, IL-6, MCP-1 and COX-2 were down-regulated in the liver of mice fed with rhubarb extract (figure 2).
Conclusions: These results suggest that rhubarb extract rich in anthraquinones might decrease liver tissue injury, namely hepatic lipid accumulation and inflammatory disorders caused by acute alcohol consumption.

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Table 1(abstract P6) Hepatic lipid contents

	Control	Ethanol	Ethanol+ Rhubarb Extract
(nmol/mg prot)			
Triglycerides	233.60 ± 18.64[a]	454.40 ± 55.65[b]	345.60 ± 32.24[a,b]
Total cholesterol	90.38 ± 8.62	161.50 ± 33.06	108.50 ± 14.45

Data with different superscript letters are significantly different at $p<0.05$ (ANOVA)

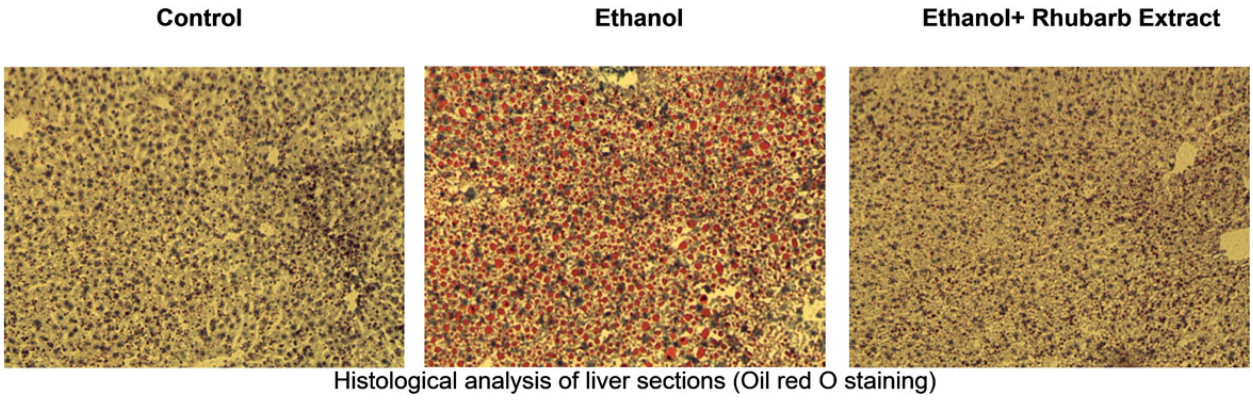
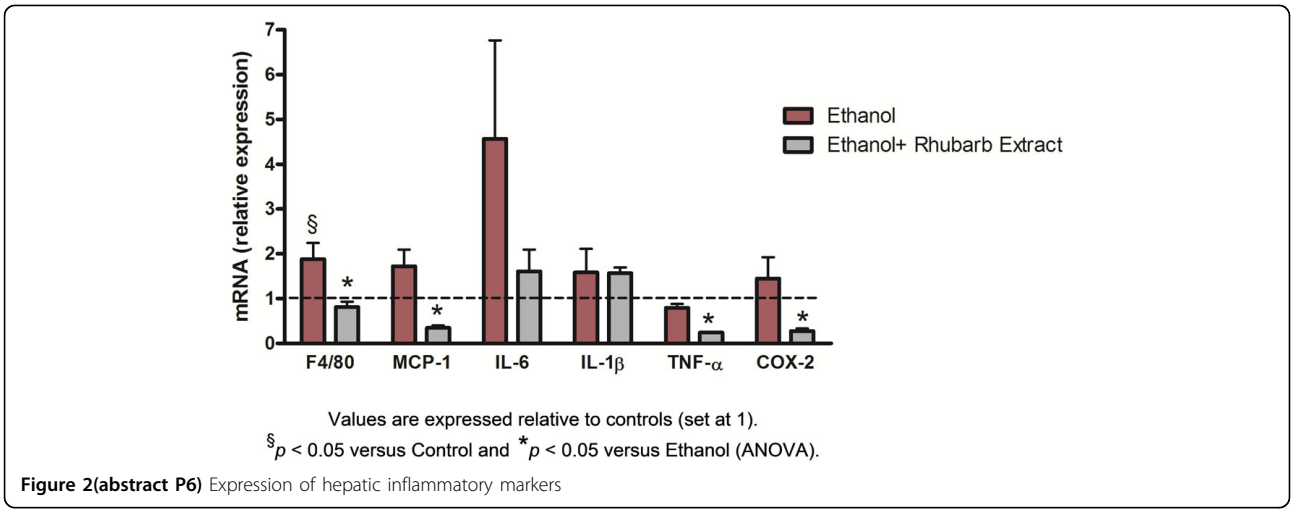


Figure 1(abstract P6) Fat accumulation in the liver



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P7

Can the supplementation of a digestive enzyme complex offer a solution for common digestive problems?

Thomas Quinten[1*], Jean-Michel Philippart[1], Thomas De Beer[2], Stefaan Vervarcke[1], Mieke Van Den Driessche[1]
[1]Metagenics Europe, Edward Vlietinckstraat 20, BE-8400 Oostende, Belgium; [2]Laboratory of Pharmaceutical Process Analytical Technology, Ghent University, B-9000 Ghent, Harelbekestraat 72, Belgium
E-mail: Thomas.Quinten@metagenics.eu
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Introduction: Proper functioning of the digestive system is imperative to assimilate nutrients, to sustain essential functions in the human body, to increase the bioavailability of nutrients, to minimize the risk of food intolerances, and to reduce the formation of toxins/irritants in the gastrointestinal-tract. Incomplete digestion often results in digestive problems such as bloating, diarrhea, stomach pain and cramps. Physicians often encounter these problems, treatment includes the use of gastroprokinetic drugs and lifestyle changes. The aim of this study was to compare the use of a gastroprokinetic agent with a full spectrum digestive enzyme complex from non-animal origin in relieving common digestive complaints.
Material and methods: An observational study was performed with 62 volunteers suffering from common digestive problems. All volunteers were eligible for treatment with a gastroprokinetic following anamnesis by a physician. Prior to the start of the study, each volunteer had to complete a validated questionnaire consisting of eight questions addressing the severity of various symptoms related to digestive disorders (0: absent, 1: low, 2: mild, 3: average, 4: severe). Then, patients were randomly assigned to a group receiving domperidone (n=19) [(Motilium®, Janssen-Cilag); dose regimen defined by physician] or an enzyme complex (non-animal origin) (n=43) [(Similase Total®, Metagenics Europe, active between pH 2-12); dose regimen: 1 capsule/meal] and treated for five consecutive days. At the end of the study, volunteers had to complete the same questionnaire and scores were collected. The mean and standard deviation were calculated and a paired two-sample t-tests was performed to investigate if significant differences were seen before and after treatment for each question in each group. In order to calculate differences between the domperidone and Similase group after treatment, unpaired two-sample t-tests were used (α=0.05). The Shapiro-Wilk test was used to assess normality.
Results: An overview of the results is presented in Figure 1. Regarding the different gastrointestinal complaints, significant improvements of all symptoms were seen following treatment with domperidone ($p<0.05$) or Similase ($p<0.05$) as evidenced by decreased scores. After five days of treatment, Similase was significantly better in reducing abdominal pain compared to domperidone (p=0.021). For the other gastrointestinal complaints, no significant differences were seen between domperidone and Similase.
Conclusion: This study showed that a digestive enzyme complex may offer a valuable alternative to gastroprokinetics to relieve various common gastrointestinal complaints.

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Risk – benefit perception and consumption of seafood in European consumers

Silke Jacobs[1*], Isabelle Sioen[2], Stefaan De Henauw[2], Núria Tous[3], Ana Luísa Maulvault[4], Gabriella Fait[5], Federico Cardona Pons[6], Wim Verbeke[1]
[1]Department of Agricultural Economics, Ghent University, B-9000 Ghent, Belgium; [2]Department of Public health, Ghent University, B-9000 Ghent, Belgium; [3]Laboratory of Toxicology and Environmental Health -Tecnatox, Universitat Rovira i Virgili (URV) School of Medicine, IISPV, 43201 Reus, Spain.; [4]Division of Aquaculture and Upgrading (DivAV), Portuguese Institute for the Sea and Atmosphere (IPMA), 1449-006 Lisbon, Portugal; [5]Aeiforia Srl, 29027 Gariga di Podenzano (PC), Italy; [6]AquaTT, Dublin 2, Ireland
E-mail: silke.jacobs@ugent.be
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Seafood consumption entails important potential health benefits such as lowering the risk of cardiovascular diseases. However, seafood may also be a source of environmental contaminants for which no maximum limits are set by authorities yet (*i.e.* priority contaminants). Exposure to these contaminants could imply health risks, especially for the more vulnerable consumer groups, such as pregnant women and children. Because of recent media attention to these contaminants, revealing the benefit and risk perception of European consumers toward seafood is of particular interest.

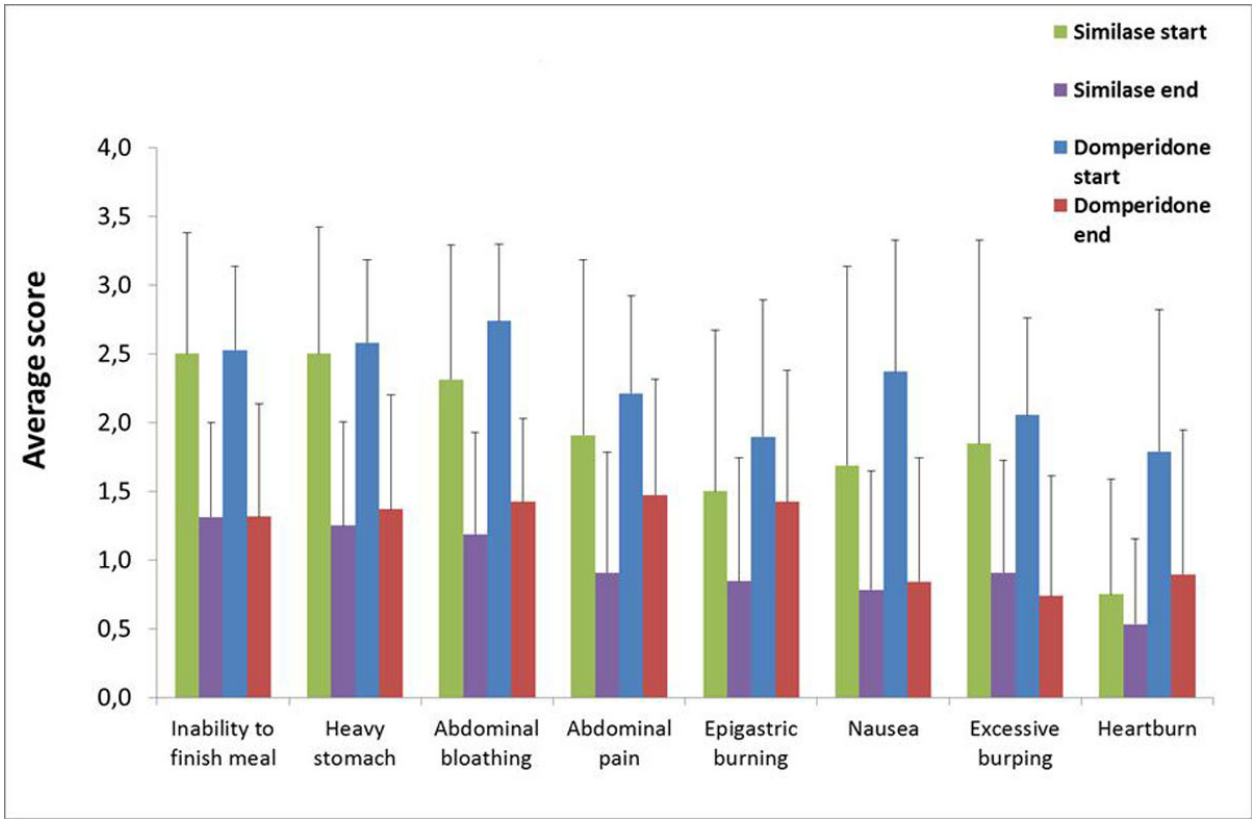

Figure 1(abstract P7) Evolution common GI-complaints.

Table 1(abstract P9) Percentiles of long-term exposure to dioxin-like compounds in adults living in Belgium, Netherlands, France, UK and Spain obtained via two classification systems

	Total dioxin-like compounds - Exposure (pg TEQ/kg bw/day)							
	Using national codes				Using FoodEx1 codes			
	P50	**P90**	**P95**	**P99**	**P50**	**P90**	**P95**	**P99**
Belgium	0.69	1.46	1.82	2.60	0.65	1.40	1.75	2.61
Netherlands	0.78	1.65	2.53	4.87	0.77	1.64	2.48	4.86
Spain	0.48	1.17	1.53	2.40	0.49	1.19	1.53	2.42
UK	0.99	1.55	1.76	2.23	0.99	1.55	1.75	2.16

For this purpose, a web based survey was performed in 2013 in five European countries, namely Belgium, Ireland, Italy, Spain, and Portugal (n=2917; age 18 to 75 years; 1451 women and 1466 men). Risk and benefit perception statements were scored on a 7-point Likert scale ranging from totally disagree to totally agree, forming a construct of seven and three items, respectively. Furthermore, consumers' concern about seafood safety was also measured on a 7-point Likert scale.

The perceived benefits of consuming seafood outweigh the perceived risks among European consumers. In general, the mean score amounts 5.43 (± 1.34) for the benefit-construct versus 2.75 (± 1.50) for the risk-construct. But, importantly, a certain concern about seafood safety has to be underlined. More in particular, 42% of the participants are concerned about the safety of seafood and 39% are concerned about the amount of environmental contaminants in seafood. It should also be noted that Portugal has the highest seafood consumption and Belgium the lowest seafood consumption with a mean self-reported consumption of 487 and 191 gram per week, respectively. Interestingly, the Portuguese consumers indicated the lowest mean score on risk perception and the highest mean score on benefit perception, both significantly different from the mean scores of the other four countries. In addition, a weak negative association is measured between risk perception and consumption ($r=-0.145$, $p<0.001$) and a weak positive association is measured between benefit perception and consumption ($r=0.214$, $p<0.001$).

Because of a potential link between risk-benefit perception and seafood consumption, it is of great interest to determine the relationship between the risk-benefit perception and consumption patterns in further analyses.

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Harmonisation of exposure assessment: a comparison between pan-European classification FoodEx-1 and national codes

Yasmina Akhandaf*, Isabelle Sioen, Stefaan De Henauw
Department of Public Health, Ghent University, Ghent 9000, Belgium
E-mail: Yasmina.akhandaf@ugent.be

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A Total Diet Study (TDS) consists of selecting, collecting and analysing commonly consumed foods purchased at retail level, processing the food as for consumption, pooling the prepared food items into representative food groups, homogenizing the pooled samples and analysing them for harmful and/or beneficial chemical substances [1]. TDSs are commonly designed at national level and aim to cover the overall diet of the population, in order to assess the dietary exposure to hazardous chemical substances of interests by the population of a certain country. The selection of food items to be analysed is based on the information available in existing consumption datasets, often on national level. To assess dietary exposure, a food classification system is needed to link existing food consumption data with the analytical data obtained in the TDS. In Europe, there is a need for a harmonized TDS approach, including a harmonised exposure assessment, to make comparison between countries possible. This study assesses the practicability of FoodEx-1, a food classification system recommended by the European Food Safety Authority (EFSA), as a classification system on pan-European level and its use for exposure assessment using TDS analytical results. The comparison was made between the exposure assessment of total dioxin-like compounds using FoodEx-1 versus national codes. This was done for five European countries; Belgium, Czech Republic, the Netherlands, Spain and the UK. The main conclusion of this study was that the exposure assessment performed with FoodEx-1 did not always accurately reflect the results of the exposure assessment obtained with national codes (table 1). However, the differences observed are minimal.

Reference

1. EFSA, FAO, WHO: Towards a harmonised Total Diet Study approach: a guidance document. *EFSA Journal* 2011, **9(11)**:2450.

Cite abstracts in this supplement using the relevant abstract number, e.g.: Akhandaf *et al.*: Harmonisation of exposure assessment: a comparison between pan-European classification FoodEx-1 and national codes. *Archives of Public Health* 2014, 72(Suppl 1):P9