Intestinal nutrient transport in the cradle of the Mediterranean Diet: 23rd EITG meeting report

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The 23rd Meeting of the European Intestinal Transport Group (EITG) was held in Salerno, Italy, on April 7-10, 2010. EITG (www.eitg.org) is an international association of scientists specifically focused on the topic of intestinal transport, both from the research and from the clinical viewpoints. EITG was created by John Robinson and Francisco Alvarado in the late 1970s with the aim of providing a friendly and informal forum for scientific discussions, giving the opportunity to young scientists to meet their seniors from all over Europe. Every other year since 1977 EITG has organized international scientific meetings in different European countries. Meeting abstracts have been published since 1978 in different Journals, and they are hosted this year for the first time in Genes & Nutrition. This supplement contains extended abstracts (including references) of the keynote lectures, and regular abstracts of the Oral and Poster contributions. All abstracts (including those of keynote lectures) are grouped according to the scientific sessions of the meeting.

The 23rd meeting returned to Italy after 15 years, and was organized by a committee composed of scientists from the National Research Institute on Food & Nutrition (INRAN) in Rome and from the Department of Pharmaceutical Sciences at the University of Salerno. The meeting was held in the Italian region Campania, an area rich of historical, architectural and natural beauties. The city of Salerno was also chosen for reasons related to science: Latin, Jewish and Arab cultures established in Salerno the first School of Medicine of the western world, the *Schola Medica Salernitana*, dating back to the IX century. Moreover, in the mid 1950s, this region greatly contributed to the first scientific reports of the health-promoting effects of the Mediterranean Diet, published by the American physiologist Ancel Keys and by his wife Margaret, who conducted their epidemiological studies on the population of Cilento, in the Salerno county (Fidanza et al. 1970). Characterized by a high cereal content (>60%), low fat (<30% - mostly monounsaturated and polyunsaturated fatty acids from olive oil and fish), high fiber (fruit and vegetables), and based on moderate but regular consumption of wine, Med Diet became the synonym of healthy diet between 1950-60. Ancel and Margaret Keys eventually moved in the late seventies from Minnesota to Pioppi, near Salerno, where they spent extensive periods of their lives until Ancel's death in 2004, at the age of 101. The healthy and sustainable Med Diet model was recently recognized by international organizations such as WHO and FAO, setting the basis for its official Nomination to UNESCO as "Immaterial Cultural Patrimony of Humanity", presented by Spain, Italy, Greece and Morocco. These aspects were further illustrated in the welcome lecture held by Giuditta Perozzi *(INRAN, Rome, Italy)* and Liberato Marzullo *(University of Salerno)*, who underlined the importance of bioactive dietary components in health maintenance and disease prevention, focusing on their enrichment in the typical food products that characterize the Mediterranean dietary profile.

The organizers dedicated the meeting to the memory of Prof. Arturo Leone, who prematurely died in 2005 (Bonatti 2006). He was a passionate scientist who devoted over twenty years of his professional life to studying the role of metal ions in human metabolism. Among the founders of the School of Pharmacy at University of Salerno, he was also among the promoters of a rebirth of the ancient School of Medicine, which had been closed in 1811 by Gioacchino Murat, King of Naples. Unfortunately, the Faculty of Medicine was

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opened only after his death, in the fall of 2006. Prof. Leone introduced at the School of Pharmacy his research activities on the molecular and cellular bases of intestinal transport and metabolism of copper and iron.

Prizes were awarded in his name to the following young scientists who presented their work at the 23rd EITG Meeting: Alejandra Pérez and Jonai Pujol *(University of Navarra, Pamplona, Spain)*, Federica Raffa *(Università di Messina, Italy)*, Matúš Soták *(Academy of Sciences of the Czech Republic, Prague)*, Evangelia Vlachodimitropoulou *(King's College, London, UK)*, Susanne M. Krug *(Charité Berlin, Germany)*, Constance Petit *(Centre de Recherche des Cordeliers, Paris, France)*. Furthermore, the Secretary General of EITG, Dr Michael Fromm *(Charité Berlin, Germany)*, awarded a special mention to the work presented by Alberto Finamore *(INRAN, Rome, Italy)*. During the meeting, recognitions were also awarded to Edith Brot-Laroche *(Centre de Recherche des Cordeliers, Paris, France)* for her longstanding contribution to EITG meetings and activities, and to Sancia Gaetani *(INRAN, Rome, Italy)*, who pioneered research on intestinal epithelial cells at INRAN over 20 years ago.

The scientific program of EITG meetings is usually structured into few Sessions, opened by plenary lectures on broad topics of special interest, followed by speakers selected from submitted abstracts. The keynote lectures of this year's meeting were especially focused on genomic technologies and on their applications in the study of intestinal physiology.

The first session of the meeting, on "Intestinal barrier physiology", was introduced by a keynote lecture by Michael Müller *(Wageningen University, The Netherlands*), who presented results from his laboratory on the role of dietary lipids in the regulation of intestinal barrier functions, investigated by using genomic approaches and transgenic animals. In particular, Dr Müller focused on the adaptive changes of small intestinal cholesterol transporters induced by high-fat diets. He also reported on the role of transcription factor PPARα as nutrient sensor and important regulator of the expression of several genes, not only involved in lipid transport and metabolism, but also in intestinal motility, nutrient transport and oxidative stress defence (Rakhshandehroo et al. 2010). The session continued with oral communications covering specific aspects of the regulation of junction complexes in the small intestine. Constance Petit (*Centre de Recherche des Cordeliers, Paris-France)* presented interesting findings on a novel role of the cellular prion protein (PrP^c) in intestinal cell-cell junction, showing that it contributes to intestinal barrier permeability by associating with and regulating assembly of desmosomal proteins (Morel et al. 2008). In addition, PrP^c deletion was shown to cause increased permeability, altered tight junction morphology and higher susceptibility to inflammation in transgenic mice. Catherine O'Neal *(University of Manchester, UK)* presented a study conducted in the differentiated human intestinal cell line Caco-2, where apical addition of some non-digestible oligosaccharides, which are part of the fibre component of the diet, led to increased tight junction permeability through a mechanism involving activation of protein kinase C and myosin light chain kinase. Expression of claudin 2, an important protein in the regulation of tight junction permeability, was reported by Svenja Plöeger (*Charité, Berlin, Germany)* to be transcriptionally down-regulated by treatment with the short chain fatty acid sodium butyrate in the intestinal cell line HT-29/B6/GR-MR, resulting in increased tightness of the junctions. Since sodium butyrate is a bacterial fermentation product, this result suggests a possible role of gut microflora in the maintenance of intestinal barrier function. On the other extreme, pathogenic bacteria such as *Yersinia enterocolica,* were reported by Nina Hering (*Charité, Berlin, Germany)* to induce barrier dysfunction in cultured HT29/B6 cells by creating "leaky areas" in the cell monolayer, in which some members of the claudin family (namely, claudin-3, -4 and -8) were re-distributed to the cytoplasm while expression of other proteins (claudin-2, -3, -8, -10 and ZO1) was down-regulated. In addition, claudin-8 activity

was shown to be regulated by the c-Jun N-terminal kinase activity (Hering et al. 2010). Aerolysin, the secreted toxin of *Aeromonas hydrophila*, was also reported by Roland Bücker (*Charité, Berlin, Germany)* to increase tight junction permeability in HT29/B6 cells, causing actomyosin contraction as well as re-distribution of tight junction proteins. In addition, the toxin was shown to affect epithelial wound healing. Both effects of the toxin could be prevented by zinc pre-treatment, which inhibits aerolysin pore formation.

Session 2 was focused on neuroendocrine regulation of intestinal metabolism. Gordon Moran *(University of Manchester, UK)* showed a positive correlation in Crohn's disease patients between post-prandial levels of the satiety-inducing enteroendocrine hormones glucagon-like peptide-1 (GLP-1) and polypeptide-Y (PP-Y) and the disease activity index, as well as with symptoms linked to the loss of appetite that characterizes this pathology. Dr Mats Jodal *(Goteborg University, Sweden*) investigated neuronal control of stem/progenitor cell renewal in mouse intestine through mucosal stimulation with the irritant capsaicin, the "hot" agent of red pepper. Proliferative cellular response within the crypts and enhanced thymidine kinase activity were shown to be nervously mediated by using neurotransmitter antagonists and selective destruction of external nerves, thus indicating that nerve cells play an important role in intestinal cell renewal and in the maintenance of intestinal barrier integrity. Bradykinin, an inflammatory mediator in the gut, was shown by Martin Diener *(Justus Liebig-University, Giessen, Germany)* to induce increased intracellular calcium in submucosal neurons from rat distal colon. The bradykinin-induced Ca^{2+} response was coupled to the $G_{q/11}$ protein but, instead of involving the classic phospholipase C signalling cascade, was mediated by an influx *via* calcium channels, probably activated by membrane depolarization resulting from inhibition of K^+ conductance. Julia Steidle (*Justus Liebig-University, Giessen, Germany)* showed an effect of carbon monoxide (CO) on anion transport in mucosa-submucosa preparations of rat colon that involved secretomotor neurons. The enzymes for CO production, hemoxigenase I & II, were detected in enteric neurons by immunohistochemistry, suggesting potential endogenous CO production. A different aspect of neuroendocrine regulation of intestinal functions was presented by Matúš Soták *(Academy of Sciences of the Czech Republic, Prague)* who reported phase-synchronized expression along the basal to apical axis of rat colonic crypts of the mRNAs encoding clock genes, the cell cycle regulator Wee1, and some ion transporters. These results indicate the importance of circadian rhythms in regulating gene expression in the intestinal mucosa, and they support the view that individual circadian clocks may control the timing of cell cycle in different regions of the gut (Polidarova et al. 2009).

The session on "Micronutrient transport and toxicity" was opened by a keynote lecture by Dennis Thiele *(Duke University, Durham, NC, USA)* who gave a comprehensive report of the mechanisms and regulation of systemic copper acquisition and mobilization in different organ systems, emerging from his studies in transgenic mice. By studying mice with tissue-specific (intestine and heart) and temporal-specific knockouts of the Ctr1 gene, encoding a Cu transporter, his laboratory could demonstrate the presence of a systemic homeostatic mechanism that allows communication of Cu status in the heart to the relevant Cu transporters in other tissues involved in Cu uptake (intestine) and storage (liver) (Kim et al. 2010). In the oral communications that followed, an interesting alternative to the treatment of iron overload with chelators was suggested in the study presented by Evangelia Vlachodimitropoulo *(King's College, London, UK)*. She showed evidence that the flavonone quercetin, already known as a substrate of glucose transporters (GLUT 1-4), as intracellular antioxidant, and as electron donor to the oxidoreductase DcytB for the reduction of Fe^{3+} to the membrane permeable Fe^{2+}, can also shuttle Fe^{2+} across the membrane via the GLUT1 transporter. Since low quercetin concentrations are orally bioavailable and non-toxic, they may be valid substitutes to the toxic iron chelators currently used in iron

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overload conditions, such as thalassemia, and following repeated blood transfusions. Chiara Murgia *(INRAN, Rome, Italy)* reported results on the tissue-specific expression and intracellular localization of the zinc-transporters ZnT4 and ZnT8. The functional form of ZnT8 in the endocrine pancreas was shown to be a highly resistant membrane associated homo-dimer (Murgia et al. 2009). Further studies using computer modelling and site-directed mutagenesis were shown, aimed at identifying specific aminoacid residues affecting stability of the ZnT8 dimers. *In vitro* studies of intestinal function frequently employ the human cancer cell line Caco-2, which undergoes spontaneous differentiation towards the phenotype of small intestinal enterocytes (Sambuy et al. 2005). However, as shown by Manuela Natoli *(INEMM-CNR, Roma, Italy)* the cell maintenance protocol (high density – HD *vs* low density - LD) can profoundly affect differentiation of these cells in terms of gene and protein expression, morphology, as well as the response to toxicants, such as Cu. In particular, cells maintained at LD were shown to exhibit more homogeneous and stronger response to Cu exposure, accompanied by increased expression of the Cu-responsive gene metallothionein 2A and of the heat shock protein HSPA1A compared to cells maintained at HD (Natoli et al. 2010). More results related to the optimization of culture conditions for differentiation of the human intestinal Caco-2 cell line were presented by Yula Sambuy *(INRAN, Rome, Italy)*. In particular, replacement of the poorly defined foetal bovine serum in the culture medium with a chemically defined supplement was shown to allow active transport of model drugs to levels similar or higher than control conditions. Emmanuelle Reboul *(Université de Mediterranée, Marseille, France)* showed that cholesterol, as well as three main dietary phytosterols, exert a strong inhibitory effect (up to 40%) on the uptake of vitamin D (cholecalciferol) in the micellar form by differentiated cultured intestinal Caco-2/TC7 cells. Although this study needs to be validated *in vivo*, it suggests that phytosterol therapy to reduce hypercholesterolaemia may adversely affect vitamin D status, which is already at risk in ageing patients. Alexandra Muscher *(University of Veterinary Medicine, Hannover, Germany)* investigated the effects of a reduced nitrogen diet (low protein) in ruminants (young goats) with the aim of lowering environmental nitrogen pollution resulting from animal feeding without affecting electrolyte balance, relying on the efficient recycling mechanisms known to be present in ruminants. However, intestinal absorption of electrolytes was shown to be affected by nitrogen feeding in goats, as it occurs in monogastric animals (Muscher and Huber 2010). A study on ruminants was also conducted by Jörg Aschenbach *(University of Veterinary Medicine, Vienna, Austria)* who showed the presence of a common bicarbonate-dependent acetate and butyrate transport mechanism in the rumen of sheep, although a bicarbonate-independent component was also detected, that was sensitive to nitrate and could be involved in acetate-butyrate exchange.

Marion Buyse *(Université Paris Sud, Paris, France)* introduced the two sessions on "Regulation of nutrient transport". She presented an overview of the role of the oligopeptide transporter PepT1 in intestinal protein digestion and of the regulation of its expression and activity by several physiological and pathological stimuli. Among these, her studies focused on the role of the leptin hormone in PepT1 regulation. While leptin secreted by stomach cells in the gastric juice promptly induces membrane insertion of PepT1 from a preformed cytoplasmic pool, leptin secreted by adipocytes regulates PepT1 at the transcriptional and translational level, followed by a time and dose dependent desensitization. In addition, PepT1 was shown to be down-regulated in two mice obesity models. The role of PepT-1 may thus extend beyond protein-absorption and may involve regulation of other nutrients for the maintenance of post-prandial energy homeostasis (references are listed in the extended abstract in this issue). A different role for leptin in the regulation of iron homeostasis was presented by Paul Sharp *(King's College, London, UK)*. Using a new co-culture system of intestinal Caco-2 cells and HuH7

hepatoma cells, the latter pre-stimulated with leptin to produce hepcidin (Chung et al. 2007), it was shown that expression of the two iron transporters DMT1 and FPN was decreased at the protein level in intestinal Caco-2 cells, paralleled by significant decrease in iron transport. The same effects were observed following direct basolateral stimulation of intestinal cells with leptin, indicating that the hormone can regulate intestinal iron homeostasis both directly and indirectly, *via* stimulation of hepcidin secretion from hepatocytes. Another transporter that undergoes rapid regulation by different stimuli is the intestinal glucose transporter GLUT2, that responds to the demand for glucose absorption through rapid intracellular trafficking between apical and basolateral membranes (Kellett et al. 2008). Maud Le Gall (*Centre de Recherche des Cordeliers, Paris-France)* investigated the relevance of GLUT2 as a marker for insulin resistance and obesity in human intestinal cells. Permanence of GLUT2 in the apical membrane and endosomal accumulation of the protein were shown to be associated with obesity or with prolonged exposure to an unbalanced high fat diet both in mice models and in humans. Richard Boyd *(University of Oxford, UK)* introduced the concept of identification of novel nutrient transporters linked to the induction of metabolic events. As an example, he described a new tryptophan transporter, distinct from classical system L activity, which was identified following induction of intracellular indoleamine 2,3 dioxygenase activity. Samyuktha Muralidharan Pillai *(Oxford Brookes University, Oxford, UK)* illustrated expression studies in *Xenopus* oocytes aimed at the functional characterization of the orphan proton–coupled amino acid transporter hPAT4. The results indicate that hPAT4 transports proline with very high affinity, but has a different range of affinities than hPAT1 and it does not exhibit the typical proton-dependence of other members within this class. Sergey Metelsky *(RAMS, Moscow, Russia)* presented some evidence, based on short-circuit current measurements, in favour of the presence of an enzyme-transport complex in rat intestine that would hydrolyze maltose and rapidly transfer the released glucose to a Na^+-dependent uptake mechanism, without releasing it into the surrounding medium. The use of thylakoids, chloroplasts membranes isolated from spinach leaves, to reduce and retard glucose uptake under conditions of hyperglycemia, was suggested by Bjorn Westrom *(Lund University, Lund, Sweden)*, based on transport studies conducted with rat intestinal segments mounted in Ussing chambers.

The intestinal microbiota has been recognized to play a fundamental role in gut physiology, influencing metabolism, immune defences and contributing to the health of the whole organism. The session on "Microbe-host interactions" was introduced by the keynote lecture of Marco Ventura *(University of Parma, Italy)* who presented recent results on the characterization of the *Bifidobacteria* component of the human gut microflora by using probiogenomics (Ventura et al. 2009). Significant inter-subject variability and composition differences between fecal and mucosal-adherent bacteria were observed. Since *Bifidobacteria* contribute to the maintenance of a balanced gut microbiota, they are frequently used as probiotics in functional foods. Genomic analysis of autochthonous *Bifidobacteria* within human intestinal microbiota contributes to the understanding of the evolutionary history within this genus, and of the metabolic mechanisms employed by commensal/probiotic bacteria to adapt to human intestinal environment (Bottacini et al. 2010). Among the health promoting effects of probiotic bacteria is their anti-diarrheal effect (de Vrese and Marteau 2007), although the mechanisms involved are still largely unknown. Pradeep Dudeja *(VA Medical Center, Chicago, IL, USA)* showed that treatment with *L. acidophilus* or with its culture supernatant induced short-term stimulation of Cl^-/OH^- exchange activity *via* increased apical levels of DRA (SLC26A3), the predominant apical Cl^-/OH^- exchanger, as well as increased expression of the mRNA for DRA and NHE3, the predominant Na^+ absorbing isoform, both in cultured intestinal Caco-2 cells and in *in vivo* mouse studies (Raheja et al. 2010). The composition of the diet can exert a

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profound effect on the intestinal microflora. Danuta Kruszewska *(Lund University, Lund, Sweden)* reported that feeding one-year-old pigs a "Paleolithic" diet, resembling the diet of our human ancestors, influenced their small intestinal microflora both quantitatively (higher bacterial numbers compared to standard diet) and qualitatively (higher proportion of anaerobes than aerobes compared to standard diet).

The meeting was closed by a session covering some aspects of intestinal physiology under pathological conditions. Marloes Schepens *(NIZO Food Research, Ede, The Netherlands)* employed HLA-B27 transgenic rats, a widely used animal model in IBD, to investigate whether antioxidant treatment (a mixture of GSH, vitamin C and vitamin E) could be protective towards this pathological condition. However, antioxidant treatment was ineffective in these rats, as they showed no evidence of oxidative stress in the colonic mucosa despite development of colitis. Exocrine pancreatic insufficiency (EPI) is frequently treated with a mixture of porcine-derived pancreatic enzymes. Stefan Pierzynowski *(Instytut Medycyny WSI, Lublin, Poland)* tested the efficacy of an alternative, microbially-derived enzyme mixture, Liprotamase, in a pig model of EPI induced by pancreatic duct ligation. He presented results indicating that Liprotamase may represent an effective alternative to traditional porcine enzyme therapy in the treatment EPI in humans, as it requires fewer tablets and it is free of zoonose risk. Alberto Finamore *(INRAN, Roma, Italy)* closed the session (and the meeting) with a report on the isolation of tissue-transglutaminase (TG2)-specific T cells from peripheral blood of coeliac disease patients and of potential coeliacs (carrying the HLA-DQ2 susceptibility haplotype). These cells were not detected in normal subjects. T-cell lines were obtained by antigen-specific stimulation and a detailed *in vitro* characterization was carried out. Incubation of mucosal specimens from treated coeliac patients with the supernatant of TG2-specific T cell clones produced characteristic disease lesions, suggesting direct involvement of TG2-specific cellular immune response in the pathogenesis of coeliac disease (Ciccocioppo et al. 2010).

Overall, the meeting was extremely interesting, and stimulating discussions were carried over from scientific sessions to the posters, meals and social events, where new collaboration were formed and old ones were strengthened. We hope that everybody enjoyed all of it, as well as…our Mediterranean food! The next appointment with EITG is for September 2011 in Oxford (UK).

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