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Abstracts and Poster

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Annals of Nutrition & Metabolism

Plenary Session

1

Pregnancy/Maternity and Child Development: The Challenge to Meet the Needs for the Brain and Sustainability of Homo Sapiens

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The brain is a fat rich system. We showed in 1972 it requires specific long chain highly unsaturated fatty acid for cell growth, structure and function: arachidonic (ArA) and docosahexaenoic (DHA) acids. The latter dominates the signaling system membranes which in the photoreceptor it is present to the exclusion of all other polyenoic fatty acids.

Vision and the brain evolved in the sea 500 million years ago using DHA. The conservation of DHA in the signaling systems of the photoreceptor and the brain over a 500 million year period of wide genomic change occurred despite the fact that the precursor differs only by 2 hydrogens and was of course easier to make and more stable. Mammals evolved on land about 100 million years ago (mya). However, 50–60 mya. land based mammals, wave after wave, moved from land to occupy the coast lines and then become fully committed back into the marine environment. It is now known that DHA is not only a key to neuronal signalling but also is a regulator of gene expression in the brain offering a cogent explanation for the emergence of H. sapiens making use of the coastal and aquatic resources rich in DHA and iodine. Both are poorly represented in the land food web.

The key to this evolutionary process would have been the women as the most critical determinants of brain development occur before birth. We described the biomagnification process whereby the proportion of DHA in the phosphoglycerides is incremented from maternal plasma to fetal cord blood, and again by the fetal liver and finally the brain. We have also described the powerful selectivity by the developing brain for DHA and synapse compared to its precursor. Yavin (Weismann Institute) and Kim (NIH) have described the implications in neurogenesis, neuronal migration and dendrite arborization. Hence, multi-generational, epigenetic enhancement led ultimately to H. sapiens.

However, I shall argue that it was not DHA alone that was responsible. ArA would have also played a significant role. About 400 mya during the Devonian period, sea creatures like Acanthostega emerged onto the land. Reproduction was by egg laying. However, 300 my. later in the Cretaceous period flowering plants with protected seeds became dominant in the wake of the collapse of the giant reptiles, gingkos, the ferns and their allies. This shift in the flora introduced quantities of linoleic acid into the land food web. With ArA as its main metabolic product offering both adhesion and angiogenesis it may be no coincidence that the fertilized egg adhered to the uterine wall with vascularization resulting in the placenta and hence the mammals. The constant perfusion and singleton products of conception dramatically changed its nutrition. Placental biomagnification for ArA is greater than for DHA. This preference is most likely for the growth of cardiovascular and placental systems needed ahead of the fetal brain growth thrust. Access to the food of the littoral system, rich in ArA, DHA and iodine would have been simple for both mothers and children. Even today after 50 my of living in the sea the grey whale muscle and liver lipids are still rich in ArA needed for mammalian reproduction.

That is maternal nutrition was a determinant of the evolution of the human brain and ultimately Homo sapiens. Recent intensification of animal and land foods with a decline in breast feeding, fish and sea foods would consequently be expected to result in a rise in brain disorders. This we predicted in 1972 and it has now happened. In the EU audit of health costs in 2004 brain disorders were found to have overtaken all other burdens of ill-health at a cost of €386 billion. In 2011 the cost was reassessed at €789 billion. The concern Governments and AID agencies have over poor nutrition, learning difficulties and behavioural disorders being directed towards children and young adults although welcome is misplaced. It is like closing the stable door after the horse has bolted. Do what you can with the child. However, the pivotal role of the mother, maternal health and nutrition needs to be recognized and central for the attack on mental-ill-health. Just as the brain evolved consequent on the appropriate biochemistry, so it can decline. The increasing brain disorders represent the most grave of challenges to the sustainability H. sapiens.

2

Nutraceutical Intervention in Children with ADHD

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There has been growing interest, from both patients and clinicians, over the past few years in the potential benefits of various nutraceuticals in the treatment of ADHD. Much of this interest has focused on the use of omega-3 to reduce ADHD symptoms. This presentation will review recent scientific and clinical studies that have investigate potential mechanisms of action and clinical efficacy of omega 3/6 fatty acid in ADHD. Recent preclinical animal studies have investigated the effects of omega-3/6 on spontaneous activity, amphetamine-induced locomotion and impulsivity (delayed discounting task) in rats. The enhanced diets appeared to have had a significant functional effect on brain dopaminergic transmission in the direction predicted by deficiency studies. These data provide preliminary support for

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the hypothesis that the omega 3/6 may have efficacy as a treatment for the hyperkinetic component of ADHD but are unlikely to influence the impulsivity seen in these patients. There are now several good quality clinical trials investigation the efficacy of Omega 3/6 in ADHD populations. In general these are positive however there are several important methodological limitations that will be discussed. Systematic and meta-analysis review does however pride some support for these agents having a role in the management of ADHD.

3

Activation of Mast Cells and Neuroglia Leads to Brain Inflammation Inhibited by Luteolin – Implications for Treatment of Alzheimer's and Autism

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New evidence suggests an association between Autism Spectrum Disorders (ASD) and Alzheimer's Dementia (AD) with brain inflammation in regions important for cognitive function [1, 2]. ASD are neurodevelopmental disorders that affect 1/88 children and are characterized by difficulties in communication, cognitive and learning defects and stereotypic behaviors. There are no reliable biomarkers or distinct pathogenesis. In spite of the use of psychotropic agents in over 70% of ASD children, recent reviews showed that they had little benefit. The market size of ASD was $15 billion in 2010 and is expected to grow 12% per year. The burden to the economy is about $35 billion to the US and it costs $3.2 million for the life-long care and treatment of each patient. AD has been associated with senile plaques and neurofibrillary tangles that involve amyloid-β (Aβ) and tau proteins. However, in spite of intensive research, clinical trials targeting Aβ have failed [3]. Recent evidence instead implicates oxidative stress/mitochondrial dysfunction [4] and brain inflammation. Presently, 1/20 individuals > 65 old has dementia and this population is expected to rise from 17.4% in 2010 to 24% in 2030 (~200M). The worldwide costs of AD will exceed 1% of global GDP, reaching $604 billion for the US.

Mast cells are important in allergic, immune and inflammatory diseases [5]. They are also critical in neuroinflammation [6, 7] and in mitochondrial health [1, 8]. Flavonoids such as luteolin and quercetin are anti-oxidant and anti-inflammatory [9]. They also inhibit mast cell activation [10] including IL-6 [11, 12], which is increased in ASD brains [13]. We showed that luteolin can inhibit mercury-induced human mast cell activation [14]. Luteolin also inhibits IL-6 release from microglia [15] is neuroprotective [16] and mimics brain-derived neurotrophic factor (BDNF) [17]. Luteolin can also inhibit mast cell-dependant auto-immune T cell activation [18], inhibits cytokine release from peripheral blood monocytes from MS patients, and may be a possible treatment for MS [19]. Luteolin is protective against amyloid β protein-induced toxicity in cultured cortical neurons [20]. Flavonoids could be useful for both neuroinflammation [21], as well as cognition and dementia [22]. Unfortunately, < 10% of orally ingested flavonoids are absorbed in powder form and are metabolized in the liver.

We developed two unique dietary supplements, NeuroProtek® and NeuroActif™ containing a patented (US patents No. 6,624,148; 6,689,748; 6,984,667; 7,115,278; 7,906,153; 12/861,152 and EPO 1365777 awarded to TCT) combination of liposomal luteolin (>95% pure from chamomile) formulated in olive kernel oil, which also increases cognition [23]. NeuroActif™ also contains hydroxytyrosol [24, 25] and oleocanthal [26], which are protective against Aβ-induced brain toxicity. Luteolin is safe [27]. An open-label case series using NeuroProtek® in children with autistic disorder (n=37) for 4 months reported (using the Global Response Assessment) that GI and allergy symptoms improved in about 75% of children, eye contact and attention in 50%, social interaction in 25% and resumption of speech in about 10%. There were no adverse effects [28]. A similar pilot open-label study of patients with Minimal Cognitive Impairment (n=13) showed significant improvement in "brain fog" and short term memory.

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4

Flavonoids and Brain Health: Multiple Effects Underpinned by Common Mechanisms

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Evidence suggests that dietary phytochemicals, in particular flavonoids, may exert beneficial effects on the central nervous system by protecting neurons against stress-induced injury, by suppressing neuroinflammation and by improving cognitive function. Historically, they were believed to do this via an ability to express classical antioxidant activity in the brain. However, their poor brain bioavailability and extensive metabolism means that this is unlikely. Instead, their actions on the brain appear to be mediated by two separate mechanisms. Firstly, they interact with critical protein and lipid kinase signalling cascades in the brain, leading to an inhibition of neurotoxin-induced apoptosis, neuroinflammation and the promotion of synaptic plasticity. For example, their ability to activate the extracellular signal-regulated kinase (ERK1/2) and the protein kinase B (PKB/Akt) signalling pathways leads to the activation of the cAMP response element-binding protein (CREB), a transcription factor responsible for increasing the expression of a number of neurotrophins critical in memory processing. Secondly, they induce effects on both the peripheral and cerebro-vascular system that lead to changes in improve blood flow to the brain capable of causing angiogenesis, neurogenesis and changes in neuronal morphology. Through these mechanisms, the consumption of flavonoid-rich foods throughout life holds the potential to limit neurodegeneration and to prevent or reverse age-dependent loses in cognitive performance. In addition, flavonoids may represent important precursor molecules in the quest to develop a new generation of brain enhancing drugs.

5

Selected Micronutrients in Cognitive Impairment with and without Dementia

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Vascular risk factors have been shown to be strongly associated with increased risk for Alzheimer's disease (AD), the most frequent form of dementia. VRF are associated with oxidative stress known to date to occur early in the onset of cognitive impairment. The reciprocal relationships between oxidative stress, vascular comorbidities and cognitive impairment progression, however, are poorly understood. Two-hundred and fifty subjects aged 65 years and older were included in the longitudinal study **O**xidative stress, **V**ascular comorbidities and their **I**mpact on **D**ementia in the elderly: description and preliminary results of the OVID* study, whose mean follow up of two years is still ongoing. Fifty patients with mild cognitive impairment (MCI group), 50 AD patients (AD group) free from VRF as well as 50 MCI and 50 AD patients with atherosclerosis, type 2 diabetes and/or congestive heart failure (groups MCI plus and AD plus, respectively) as well as 50 controls underwent physical examination, ECG, nutritional assessment, carotid duplex sonography and a battery of neuropsychological tests including MMSE, DemTect, Clock Drawing Test and ADAS-Cog. At baseline, patients and controls underwent a blood withdrawal for the HPLC analysis of retinol, carotenoids including lutein, zeaxanthin, β-cryptoxanthin, lycopene, α- and β-carotene, tocopherols including α- and γ-tocopherol as well as for ELISA measurements of protein carbonyls and GC/MS analysis of F2-isoprostanes. The evaluations are being performed yearly in all subjects. There were no statistically significant differences among groups as far as age, gender and BMI are concerned. MCI Plus and AD Plus patients had significantly higher carotid intima-media thickness and higher glycemic values than MCI, AD and control subjects ($p < 0.001$). MCI, AD and control subjects had significantly higher plasma levels of lutein, zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, β-carotene, α-tocopherol, γ-tocopherol and retinol than MCI Plus and AD Plus patients (min. $p < 0.01$). Results were independent of age, gender, BMI as well as intake of fruits and vegetables. In a subset analysis of

13 subjects with MCI, 19 AD, 11 MCI Plus and 23 AD Plus, baseline plasma levels of F2 isoprostanes as biomarkers of lipid peroxidation were in average 20% higher in MCI Plus and AD Plus patients whose neuropsychological scores worsened after two years than F2 isoprostane plasma levels in MCI and AD patients neuropsychologically stable at follow up. Vascular comorbidities and risk factors should be always identified in aged patients with cognitive impairment. This might lead to a better lifestyle- and nutrition-related counseling of patients with cognitive impairment and their caregivers.

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6
Update Vitamin D

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Increasing data suggest that higher 25-hydroxyvitamin D (25(OH) D) serum concentrations are advantageous for health, and may prolong healthy life expectancy. At present, strong evidence for causality is available for fracture and fall prevention, while promising epidemiologic and mechanistic studies suggest a key role of vitamin D in the preservation of cardiovascular health, and the prevention of cancer, and other common chronic disease.

For musculoskeletal health, cardiovascular health and cancer prevention, epidemiologic studies suggest a dose-response relationship with 25(OH)D levels of 75 nmol/l to approximately 110 nmol/l conferring the lowest risk of disease. Current recommendations of 800 IU per day should reduce hip fracture risk and falls by about 30%, and will shift most individuals out of vitamin D deficiency (< 50 nmol/) as a first important public health goal. Doses that will shift most individuals to a range of 75 to 110 nmol/l are less well defined and need further investigation and confirmation in large clinical trials. Notably, however, based on RCT data among the senior population, the 75 nmol/l threshold is supported by the International Osteoporosis Foundation, the US Endocrine Society and the Swiss BAG for optimal fracture reduction among individuals age 60+.

Most vulnerable to low vitamin D levels are elderly, individuals living in northern latitudes with prolonged winters and thus low UVB exposure, obese individuals, and African Americans of all ages.

7
Vitamin D Status: A Global Systematic Review of Vitamin D Serum Levels

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Vitamin D is best known to play an important role in bone metabolism and health throughout life. However, recent research has demonstrated a number of non-skeletal benefits of vitamin D for human health. The effect of vitamin D on muscle strength and falls is one of those intriguing findings which have resulted in a health claim issued by the European Food and Safety Agency (EFSA). In addition, there is a wealth of publications reporting beneficial effects of vitamin D on various other health outcomes such as hypertension, diabetes, immune status to name a few. So, the obvious question is: Do we get enough of vitamin D? To address this question we reviewed the literature reporting 25-hydroxyvitamin D (25OHD) blood level, an established marker to assess vitamin D status: 25OHD levels below 25 nmol/l are considered a deficiency, 50 nmol/l is suggested by the Institute of Medicine to be adequate to meet the requirements of 97.5% of the population and clinical studies using fracture as an outcome support a desirable 25OHD level of above 75 nmol/l. Databases searched are PubMed, Medline and EMBASE for publications in English between January 1990 to February 2011. The analysis involves close to 200 studies from more than 40 countries around the globe. More than 88% of the included population had mean 25OHD level below 75 nmol/l, 37% below 50 nmol/l and close to 7% below 25 nmol/l. This affects both the developing and the industrialized world. Besides adults, widespread gaps exist especially in children and adolescents and the prevalence of vitamin D deficiency is higher amongst women than men. For some regions such as South America and Africa published data on 25OHD levels are limited. The outcome of this systematic review is visualized in a color-coded global interactive map. The options to improve the low vitamin D status in people will be discussed and a call to action is suggested.

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8
Antioxidants as Bioactives

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The concept of biological antioxidation has evolved through several stages. Tocopherols (Vitamin E) and ascorbate (vitamin C) have been demonstrated to operate as biological antioxidants by direct redox reactions with suitable biomolecules. The European Food Safety Authority (EFSA) has concluded that vitamin E functions physiologically as a chain-breaking antioxidant that prevents the

propagation of lipid peroxidation, and that a cause and effect relationship has been established between the dietary intake of vitamin E and protection of DNA, protein and lipids from oxidative damage [1]. Likewise, a similar claim on vitamin C has been assessed with a favourable outcome [2].

However, research in recent years has made clear that small molecules which *in vitro* can serve as antioxidants may *in vivo* exhibit other functions which might even qualify as biologically more important functions. These functions include the recruitment of transcription factors leading to enhanced gene expression of a pattern of protective antioxidant enzymes. Thus, so-called antioxidant molecules may exhibit prominent antioxidant capacity in cells indirectly rather than directly. Such molecules should therefore preferably be considered as bioactives.

This conclusion is chemically plausible as the catalytic ('repetitive') function of an enzyme outweighs a stoichiometric ('one shot') reaction of a small molecule. Strategies of antioxidant defense [3], therefore, are foremost of enzymatic nature, and regulation of enzyme patterns by small molecules, antioxidant vitamins and other micronutrients has been unraveled. Most prominent is the Nrf2/Keap1 system discovered by Yamamoto's group [4] which operates through the antioxidant response element (ARE, EpRE).

It appears important to emphasize that antioxidant function of many small molecules is indirect rather than direct. A consequence of this is that assessment of *in vivo* conditions by artificial small-molecule tests, 'total antioxidant capacity'(TAC), should be discouraged [5]. When assayed in extracts *in vitro*, micronutrients of plant origin such as flavonoids can provide high scores in such assays. However, when these compounds are ingested orally, those molecules which will be taken up in the gastrointestinal tract will undergo extensive metabolism in the gut and in the liver. Flavonoids such as the flavanol, (-)-epicatechin, circulate in blood plasma in the form of metabolites (glucuronides, sulfates, methylated products), not as the free compound [6]. This means that the reactive hydroxyl groups which bear the antioxidant function *in vitro* are shielded, so that there is very little, if any, contribution to antioxidant potential within the plasma *in vivo*. In line with this, the biological relevance of direct antioxidant effects of polyphenols for cardiovascular health in humans is not established [7]. The mechanisms by which flavanol-rich foods protect against vascular dysfunction and oxidative damage have recently been discussed at the 27th Hohenheim Consensus Conference [8].

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9

Health Benefits of Mixed Fruit and Vegetable Concentrates

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There is much scientific evidence indicating that diets rich in fruit and vegetable (FV) are associated with healthy body weights and reduced risk of cardiovascular disease (CVD), diabetes and certain cancers. FV contain a variety of bioactive components (vitamins, sterols, phenolic compounds and fiber) that may protect against various diseases independently. Despite these health benefits and numerous public health campaigns to increase FV consumption, the vast majority of individuals do not meet intake levels set by national guidelines. FV concentrates, prepared from mixed FV, may represent a practical approach for individuals to improve their intake of FV and dietary bioactive compounds. A number of clinical studies have examined the effects of commercially available mixed FV products on various health outcomes. However, the possible health benefits of FV supplements have not been systematically reviewed. We recently undertook a systematic search of MEDLINE and EMBASE databases to identify clinical interventions that examined the effect of commercially available concentrated mixed FV supplements on health. Twenty-two reports, that used commercially available products, were identified. None of the studies reported any serious adverse effects. Overall, daily consumption of FV concentrates significantly increased serum concentrations of the major antioxidant provitamins and vitamins found in plant foods (β-carotene, vitamins C and E) and folate. Functional changes, such as reduced serum homocysteine and markers of protein, lipid and DNA oxidation were also reported. In addition, health advantages on markers of inflammation, immune function and endothelial function appear promising.

In summary, mixed FV supplements may serve as an efficacious complement for individuals who have difficulty achieving their daily fruit and vegetable intake requirement. Mechanistic studies and larger, randomized, placebo controlled double blind trials in both healthy and high-risk populations should be undertaken to better understand the health effects of these supplements.

10

Probiotic Efficacy in Clinical and Food Application – From Concepts to Mechanisms

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The human body can be considered as a meta-organism made up by its own eukaryotic cells and trillions of microbes that colonize superficial body sites such as the skin, the airways and the gastrointestinal tract. The coevolution of host and microbes brought a variety of molecular mechanisms about, which ensure peaceful relationship. There is a vast array of immune-mediated and metabolic-associated diseases which were found to correlate to disturbed intestinal functions and changes in the intestinal microbiota. The term "probiotic" originates from the greek expression "pro bios" (for life). Fermentation of milk products by lactic acid bacteria was used for thousands of years in many cultures to prevent rotting of food. The direct positive effect of live bacteria within these products was recognized in the 19th century and this knowledge was used to treat diarrhea. In 2001, the World Health and Food and Agriculture Organization defined probiotics as microorganisms that, when administered in adequate amounts, confer a health benefit to the host. Despite an extensive bulk of experimental data and an array of clinical studies proving that specific probiotics might be a good alternative or adjunct therapy, probiotics are far from being used as standard medication. Inflammatory bowel diseases (IBD), comprising the idiopathic pathologies ulcerative colitis (UC) and Crohn´s disease (CD), are a cluster of immune-mediated chronic disorders of the gastrointestinal tract. The etiology of IBD is a paradigm for chronic disorders of industrialized countries still characterized by increasing numbers in incidence and prevalence. Genetic susceptibility and environmental factors are crucial parameters in the pathogenesis of IBD. Clinical data and experimental studies revealed that the intestinal microbiota plays a major role in the progression of the chronic intestinal inflammation, suggesting probiotics as a reasonable therapeutic concept. Nevertheless, there are several reasons for a limited therapeutic efficacy of probiotics including uncertainty with regard to strain selection as well as the timing, duration and dosage of probiotic application in the context of the different IBD indications (UC/CD/pouchitis, acute inflammation, remission, location and severity of the inflammation). In addition, there are safety concerns regarding the application of huge amounts of living bacteria in IBD patients that are characterized by immune dysregulation and compromised intestinal barrier functions. Besides the lack of extensive and reliable clinical data with regard to these questions, all these problems burn down to the lack of mechanistic understanding of probiotic efficacy. In contrast to the use of defined pharmaceuticals like corticosteroids, NSAIDs or biological agents that are known or even designed to target a specific host structure, the protective structure function relationships underlying the observed anti-inflammatory effects of probiotics are largely unknown. In order to draw a clinical benefit of the already available promising data it is therefore necessary to reveal probiotic structure function relationships which might enable the targeted use of probiotics or isolated probiotic structures in the future.

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Future Directions for Nutritional and Therapeutic Research in Omega-3 Fatty Acids

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Omega-3 fatty acids (O3FA) are a family of polyunsaturated fatty acids that contribute to human health and well-being. Functionally the most important O3FA appear to be EPA and DHA found in oily fish and in supplements. Intakes of EPA and DHA are typically low and much below recommended intakes. Increased intakes are reflected in greater incorporation into bloods, cell and tissue pools. Increased content of EPA and DHA can modify the structure of cell membranes and also the function of membrane proteins involved as receptors, signaling proteins, transporters and enzymes. EPA and DHA also modify the production of lipid mediators and through effects on cell signaling can alter patterns of gene expression. Through these actions EPA and DHA act to alter cellular responsiveness in a manner that seems to result in more optimal conditions for growth, development and maintenance of health. The effects of O3FA are evident right through the life cycle meaning that there is a need for all sectors of the population to increase the intake of these important nutrients. However it seems that the amounts of O3FA required to achieve some benefits are quite high and will not be easily reached in the absence of extremely frequent oily fish intake or the use of concentrated oils. This presents a challenge for incorporation of EPA and DHA into foods. Solving this issue could be helped by identification of whether EPA or DHA is more important for structure and function; certainly these two related O3FA have some different effects and different potencies and should not be regarded as being equivalent. However it is not yet possible to unequivocally favour one of these O3FA over the other apart from the role in early neural and visual development where a supply of DHA seems to be vital. Two related issues are whether other O3FA are functionally important and whether certain formulations of O3FA may offer an advantage. With regard to the first of these issues, DPA is an O3FA that may have functional properties that may be shared in part with EPA and DHA and certainly require more extensive exploration. The precursors of EPA, alpha-linolenic acid (ALA) and stearidonic acid (STA) have been explored for functional effects. These plant O3FA can mimic some of the effects of EPA and DHA, but require high intakes to do so. This seems likely to relate to the need to convert ALA and STA to EPA, in which case STA is superior to ALA, although sources of STA are less common. With regard to the second issue, there is emerging evidence that the chemical formulation of O3FA may impact its effects. It seems that presenting O3FA as phospholipids may have better efficacy than presentation in other forms, although the reason for this is not yet clear. Finally, there is increasing evidence that O3FA metabolism, status and function are determined partly by genetic variations among individuals. This suggests that different genetic sub-groups of the population will have different needs for exogenous O3FA and will respond differently to a certain intake of O3FA. While the prospect of personalizing O3FA supply for achieving health and for reducing risk of or treating disease is an exciting development, this approach is clouded by a number of complex issues.

Poster

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Oxidative Status in Osteopenic and Osteoporotic Postmenopausal Women

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Little information is available about the possible role of oxidative stress (OS) on the pathogenesis of osteoporosis and its preceding condition, osteopenia, both characterized by lower bone mineral density (BMD) than normal. After menopause, bone turnover acceleration induces an imbalance between bone resorption and formation leading to final bone loss.

To study OS in osteopenia and osteoporosis, BMD of 61 consecutive women (aged 61.01±7.82; BMI 25.39±3.71 Kg/m^2) was assessed by using phalangeal ultrasonographic examination (DMB Sonic Bone profiler, IGEA, Italy); OS was assessed by evaluating serum Reactive Oxygen Species levels (ROS; cut-off<300 UCarr) and Total Antioxidant Capacity (TAC; cut-off>350 µmol HClO/mL) both by spectrophotometric methods (Diacron International, Italy) and oxidized LDL levels (oxLDL; cut-off<70 U/L) by ELISA method (Mercodia, Sweden).

Forty-four subjects (aged 59.63±7.14) were osteopenic, 17 (aged 66±6.88) osteoporotic; ROS levels were significantly higher in osteopenic group than in osteoporotic (417±77 vs 360±47 UCarr; p=0.001, respectively) while oxLDL levels were higher in osteopenic than osteoporotic subjects but not significantly so (81±35 vs 75±29 U/L); no significant difference in TAC was found between the groups (401±45 vs 398±28 µmol HClO/mL).

Osteopenic subjects showed increased OS, possibly due to osteoclastic activity, higher in osteopenia than in osteoporosis. A balance between oxidants/antioxidants is important for maintaining a correct equilibrium between osteoblastic and osteoclastic activities and regulating bone resorption.

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Probiotic Supplementation Affects Markers of Intestinal Barrier, Oxidation, and Inflammation in Trained Men

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Aim: To assess the effects of probiotic supplementation on intestinal barrier function, oxidative stress, and inflammation.

Methods: In this randomized, double-blinded, placebo controlled trial 23 trained men received 10bn CFU/day of multispecies probiotics (ECOLOGIC®Performance/in A, CH, D: OMNi-BiOTiC®POWER) or placebo. They performed an intense exercise test at baseline and after 14 weeks of treatment. Zonulin and α1-antitrypsin from feces were measured at baseline and after 14 weeks to estimate intestinal barrier function. The following blood parameters were measured before and after each exercise test: carbonyl proteins (CP), malondialdehyde (MDA), total oxidation status (TOS), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6).

Results: Zonulin decreased after 14 weeks of probiotic supplementation from values above normal into normal ranges and was significantly lower in the probiotic group compared to placebo (p=0.019). CP increased significantly from pre to post exercise in both groups (p=0.006). Probiotic supplementation decreased pre and post exercise CP concentrations resulting in a difference by a trend (p=0.061). TOS was slightly increased above normal in both groups, at baseline and after 14 weeks. There was no effect of supplementation or exercise on TOS. At baseline, both groups showed considerably higher TNF-α concentrations than normal. After 14 weeks TNF-α was reduced in the supplemented group and tendentially lower in comparison to the placebo group (p=0.054). IL-6 increased significantly post exercise in both groups (p=0.001), but no effect of supplementation was observed. MDA and α1-antritrypsin were not influenced, neither by supplementation nor by exercise.

Conclusions: Probiotic treatment decreased zonulin in faeces, a marker indicating enhanced gut permeability. Moreover, probiotic supplementation beneficially affected TNF-α and protein oxidation. These results demonstrate promising benefits for probiotic use in trained men.

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Natural Antioxidant Activities of Phellinus Mushroom Extracts

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Antioxidants are considered important nutraceuticals on many health benefits. Phellinus is a genus of fungi in the family Hymenochaetaceae. Phellinus mushroom is commonly used in traditional herbal medicine for treatment of various diseases in Asia. The purpose of this study was to investigate the antioxidant activities of the extracts from Phellinus mushrooms. The three species of Phellinus mushroom, which are Phellinus rimosus, Phellinus wahlbergii, and Phellinus nigricans, were interested for this study. Each Phellinus mushroom was extracted by three methods i.e. maceration in water and ethanol, and an extraction procedures for alkaloids [1]. The crude extracts of Phellinus mushroom were investigated the antioxidant activities. The ascorbic acid was used as a reference standard. Two measurements of antioxidant activities were employed using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging assay [2] and the ferric reducing antioxidant power (FRAP) assay [3]. The results showed that the alkaloid extract from Phellinus nigricans possessed the strongest antioxidant activity in the DPPH radical scavenging assay with an EC50 value of 7.03±1.76 μg/mL whereas EC50 value of ascorbic acid was found at 5.02±0.46 μg/mL. In addition, the ethanol extract of Phellinus nigricans showed the highest FRAP value of 0.33±0.01 mM Fe(II)/mg in FRAP assay whereas FRAP value of ascorbic acid was found at 0.33±0.02 mM Fe(II)/mg. In conclusion, the results suggested that the Phellinus extracts showed potential antioxidant activities to develop as a nutritional supplement for prevention of some aging diseases caused by free radicals.

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Investigating the Biochemical Background for Histamine Intolerance: Degradation Capacity of Mixtures of Biogenic Amines by Human, Porcine and Bovine Diamine Oxidase

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Diamine Oxidase (EC 4.1.3.22; DAO) is accepted to be the main enzyme for degradation of foodborne biogenic amines in the intestine [1–3]. Until now little data of total degradation capacity of biogenic amines of DAO are available. We have purified DAO from porcine and bovine kidney and from human placenta using a two dimensional chromatographic process. Enzyme purity was shown to be > 90%. For the determination of enzyme activity the hydrogen peroxide produced during catabolism of biogenic amines was detected in a modified fluorescent method [4]. Detection limit of this method was 20 nmol/ml. To determine the total biogenic amine degradation capacity (TBADC) DAO was incubated with Histamine, Putrescine, Cadaverine, Spermine, Spermidine and Serotonin respectively. Substrates were tested in various concentrations between 7 μM and 5 mM. Additionally mixtures of these biogenic amines were tested as substrate. Results: relative reactivity of h-DAO was 100%, 42%, 32%, 5% for putrescine, cadaverine, histamine and spermine respectively. Substrate inhibition occurred at 450 μM, 1000 μM, 50 μM for putrescine, cadaverine, and histamine respectively. Degradation of histamine was reduced to 60% in presence of 100 μM putrescine or cadaverine. Degradation of putrescine was reduced to 68%, 46% and 33% in presence of 50, 150 and 450 μM histamine respectively. Degradation of cadaverine was reduced to 66%, 52% and 42% in presence of 50, 150 and 450 μM histamine respectively. Conclusio: physiological histamine degradation by DAO is influenced significantly in the presence of e.g. putrescine or cadaverine. Therefore not only the presence of histamine, but also the content of other biogenic amines in food has to be considered for the evaluation of a diet. As a consequence the symptom complex should be named

"Biogenic Amines Intolerance Syndrome" (BAIS) rather than "histamine intolerance" (HIT) to fully contribute to the biochemical background of the syndrome.

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Anticancer Activity of Raw and Digested Irish Palmaria Palmata Seaweed on In Vitro Models of Colorectal Cancer

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Purified seaweed compounds have been shown to reduce cancer cell proliferation in vitro and in vivo [1, 2]; however the anti-cancer characteristics of consumed, crude seaweed are not well known. The aim of this study was to investigate the anti carcinogenic potential of Irish seaweed Palmaria palmata pre- and post-simulated gastric digestion on in vitro models of colorectal cancer.

Two extracts were prepared from fresh, Irish Palmaria palmata samples: a "crude" homogenate and a "digested" fraction, which represented colon available material after in vitro simulation of human gastro-intestinal digestion. The anti-proliferative activity of the extracts was tested in vitro using three colorectal cancer cell lines: HT29 early adenocarcinoma, $CaCo_2$ adenocarcinoma and HT115 metastatic carcinoma. An MTT cytotoxicity assay was used to establish a sub-lethal dose range of treatments on all cell lines. The anti genotoxic potential of the seaweed extracts was assessed using a COMET assay and anti-proliferative activity was tested using 7 day cell growth experiments. The anti-metastatic potential of extracts was estimated by measuring the inhibition of cellular invasion and migration in vitro.

Results indicated that in vitro gastro-intestinal digestion reduced the content of biological compounds in the extract; however the antioxidant capacity was not greatly affected. Both crude and digested extracts were toxic to tested cell lines at doses ≥10 mg/ml dry weight. Seaweed extracts exhibited anti-proliferative activity in a dose-dependent manner during 7 day growth measurements. Anti-genotoxic effects were observed with 3.75–5 mg/ml raw extract treatments (27–32% DNA damage reduction), but not with digested extract. Both crude (2.5–5mg DW/ml) and digested (5–10mg DW/ml) extracts inhibited invasion and migration of metastatic HT115 cells. Normalized to untreated control the reduction in invasion was 36–72% and in migration 28–65%.

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Effects of a Juice Powder Concentrate and Walking Exercise on Systemic Markers of Oxidation, Inflammation and Skin Microcirculation in Obese Women

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Introduction: In recent decades increasing prevalence of obesity has become a serious public health concern. Obesity is associated with irregularities in redox homeostasis, imbalanced pro-inflammatory and anti-inflammatory states and microcirculatory dysfunction [1–3].

Aim: To assess the impact of 8-wk supplementation with the encapsulated juice powder concentrate or placebo, and a single bout of controlled walking on markers of oxidation, inflammation, and microcirculation in the skin of obese women.

Methods: We monitored 42 premenopausal (41±5 y, non-smokers, BMI 34.5±3.8) women using a randomized, double-blinded, placebo-controlled design; before and after 8 wk of treatment; and, before and after a 30 min controlled treadmill walking test at 70% of VO2max. Venous blood for determination of carbonyl proteins (CP), malondialdehyde (MDA), oxidized LDL (oxLDL), total oxidation status of lipids (TOS), IL-6 and TNF-α was collected before and immediately after exercise. Pre- and post-exercise, the parameters of skin microcirculation, oxygen saturation of hemoglobin (SO2Hb), relative concentration of hemoglobin (rHb), and blood flow were assessed at 2mm depth from the skin surface.

Results: After the 8-wk supplementation, the FBV group showed significant ($P<0.05$) lower concentrations of CP, oxLDL, TOS and TNF-α compared to placebo. Further, we observed significant higher values of SO2Hb, rHb and blood flow in the FBV group. 30 minutes of walking exercise also significantly increased rHb and blood flow with a trend toward increased SO2Hb.

Conclusion: These data indicate that the nutraceutical decreased plasma oxidation and inflammation status and improved microcirculation at the skin surface. Exercise also improved skin microcirculation and blood flow under the skin. The study also demonstrates a better beneficial outcome on redox homeostasis and skin microcirculation in overweight and obese women when both factors, supplementation and exercise, are combined.

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Modulation of the Production of Inflammatory Metabolites in THP-1 Derived Macrophages

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Background: Macrophages and their chemical mediators play a pivotal role in the autoregulatory loop of an inflammatory process. Specialized lipid mediators are synthesized from fatty acids released from inflammatory cell membranes. Previous studies have shown that fatty acids have different effects on inflammatory signalling pathways [1]. This study aims to further explore the changes in gene expression and enzymatic signaling induced by LPS and fatty acids and how these changes orchestrate the synthesis of lipid mediators and cytokines.

Methods: THP-1 cells were cultured with supplemented RPMI 1640 medium and differentiated to macrophages using phorbol 12-myristate 13-acetate (PMA). Macrophage phenotype was confirmed by surface protein expression and morphology. THP-1 derived macrophages were incubated with different concentrations of LPS for different times or fatty acids for 12 h. Cell pellets were collected for analysis of gene and protein expression. Lipid mediators and cytokines released in cell supernatants were evaluated by mass spectrometry and flow cytometry, respectively.

Results: PMA treatment induced the expression of macrophage features on THP-1 cells. The expression of COX, LOX and INOS were differently affected by LPS treatment. Lipid mediator levels were altered during LPS treatment and followed a pattern similar to that of some of the cytokines. The effect of LPS and fatty acids on gene expression will be presented.

Conclusions: Our results suggest that the production of cytokines and lipid mediators is regulated by NFkB and other transcription factors may be involved. These signalling pathways may also explain COX, LOX and iNOS expression levels. Fatty acids may induce changes in inflammatory genes and signaling pathways in THP-1 macrophages.

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Chemical Constituents and In Vitro Anticancer Activity of Tiliacora Triandra Leaves

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Tiliacora triandra (F. Menispermaceae) is a well-known edible and medicinal plant in Thailand. Leaves of the plant are cooked with bamboo shoots in the northeast of Thailand [1]. The aims of this study were to (a) determine the major constituents of these leaves and (b) assess their anticancer activity against human cell lines. The leaves were extracted by a soxhlet apparatus with petroleum ether, dichloromethane, ethyl acetate and macerated in water. Major constituents were then purified and identified using chromatographic procedures and various spectroscopic techniques. In vitro anticancer activity was tested using the resazurine ELISA microplate assay (REMA) and oral cavity cancer (KB), lung cancer (NCI-H187) and breast cancer (MCF-7) cell lines [2]. The results showed that the main compound present in the methanol extract wase oxoanolobine. The water extract exhibited the highest anticancer activity against the KB cell line, whereas the methanol extract was most effective against the NCI-H187 cell line. The IC50 of oxoanolobine against the NCI-H187 cell line was 27.60 ± 4.30 μg/ml. Further research is needed but results presented here suggest that T. triandra represents a functional food for health promotion.

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The Impact of a 'Probiotic' Modulation of the Gut Microbiota on Hepatic and Peripheral Insulin Sensitivity in Human Subjects

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Recent animal experiments suggest that ingestion of pre- or probiotics influences gut microbiota composition and intestinal permeability as well as insulin resistance, components of the metabolic syndrome and diabetes development [1–5]. In humans, faecal transfer from lean into obese subjects was reported to improve insulin sensitivity [6]. We tested the hypothesis that Lactobacillus reuteri enriched microbiota improves insulin sensitivity and secretion as well as glucose tolerance in lean and obese healthy humans by improving gut hormone secretion.

We performed a double-blind, randomized, prospective, longitudinal parallel group trial in 18 glucose tolerant participants (9 obese: mean age 51±2 years, body mass index 36±5 kg/m^2 and 9 lean: 49±4 years, 24 ± 2 kg/m^2). Subjects ingested 2 capsules/day containing placebo or 1010 L. reuteri (Nutraceutix, USA) for 4 consecutive weeks. Identification of L. reuteri in faecal probes via qPCR and capsule counting monitored compliance. Whole-body (peripheral) and hepatic insulin sensitvity were assessed using the euglycemic-hyperinsulinemic clamp combined with stable isotope dilution of 6,6[2H2] glucose. The incretin-mediated effect on insulin secretion was evaluated by isoglycemic intravenous glucose infusion tests in comparison to oral glucose tolerance test. Ectopic fat content in liver and muscle was measured by MR spectroscopy.

Peripheral insulin sensitivity was lower in obese than in lean humans ($p<0.01$). Basal endogenous glucose production was suppressed by insulin in obese (31%) and lean participants (47%, $p<0.01$). Ingestion of L. reuteri neither affected body weight, mass nor ectopic fat content. Likewise, peripheral and hepatic insulin sensitivity (37% vs. 38% suppression) remained unchanged. In conclusion, daily ingestion of L. reuteri enriched gut microbiota but had no effects on insulin sensitvity in non-diabtetic humans. These results will be of interest in the acute use of probiotics in insulin resistance.

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Resveratrol Induces Potent Effects in the Activity of Primary Cortical Networks Cultured on Microelectrode Array Neurochips

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Microelectrode array (MEA) neurochips with primary neuronal networks from embryonic mice can be used to study the effects of neuro-active compounds through their changes of the electrophysiological activity patterns in a qualitative and quantitative manner [Gramowski et al., 2006].

We tested Trans-resveratrol, Polygonum cuspidatum extract containing 50% Trans-resveratrol and an Anthraquinone fraction of the Polygonum cuspidatum extract. These extracts were applied to frontal cortex networks in a cumulative increasing manner to the screening setup.

We recorded the extra cellular action potentials from the MEA neurochips at baseline and following compound application. The time stamps of the action potentials, also called spike trains, are analyzed by sophisticated algorithms. Up two 200 parameters are calculated for each spike train, allowing us to determine with high sensitivity, functional effects of compounds on the network activity regarding changes in the general activity, burst structure, synchronicity and oscillatory behavior.

All three extracts induced potent effects on cortical network activity. The half effective concentration EC50 for the parameter spike rate (number of action potentials per time) was 117 pg/ml for Anthraquinone, 32.6 ng/ml for Polygonum cuspidatum extract containing 50% Trans-resveratrol and 20.4 pg/ml for Trans-resveratrol.

Effects of Trans-resveratrol and Anthraquinone on other spike train parameters indicate that both extracts decreased the strength of the burst activity, revealed by a decrease in the burst structure parameters burst duration, peak spike frequency, and spikes in burst, as well as the general activity parameter percentage of spikes grouped in bursts.

The studied compounds showed clear and potent effects on primary neuronal network cultures. These data can deliver a rationale for observed effects of especially Resveratrol on the brain.

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Antioxidant and Antimutagenic Activities of Extracts and the Major Constituent, Syringic Acid, of Ardisia Elliptica Fruits

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Ardisia elliptica (AE) fruits are used in Thai traditional medicine and herbal wine. The aims of the study were to evaluate antioxidant and antimutagenic activities of AE fruit extracts and an isolated major constituent. Chromatographic purification and spectroscopic structure studies provided the main active compound, namely syringic acid [1]. Antioxidant activity was expressed as the ability of each extract to scavenge the free radicals 1,1-diphenyl-2-picrylhydrazyl (DPPH) [2]. Antimutagenic activity was evaluated in accordance with the Ames test [3] using Salmonella typhimurium strains TA 98 and TA 100 with concentrations from 12.5–200 μg/plate. The results showed that syringic acid possessed the strongest antioxidant activity with the EC50 values at 0.24 ± 0.04 μg/mL. Furthermore, syringic acid also showed the strongest antimutagenic activity with both strains of S. typhimurium with percentage of inhibition values ranging from 90.94 ± 0.48 to 99.50 ± 0.49. With regard to AE fruit extracts, methanol extract showed the highest antioxidant activity with EC50 at 8.87 ± 0.24 μg/mL, followed by dichloromethane extract (14.24 ± 0.04 μg/mL) and hexane extract (50.01 ± 0.56 μg/mL). In addition, antimutagenic activity of dichloromethane extract exhibited the highest percentage of inhibition, followed by hexane extract and methanol extract, respectively. The apparent antioxidant and antimutagenic activities of the AE fruits extracts suggest its potential usefulness for health promotion.

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The Effect of Daily Oral Intake of Probiotics on the Frequency and Intensity of Migraine Attacks – A Pilot Study

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Migraine is a neurogenic inflammatory disorder. The presence of migraine is associated with multiple gastrointestinal diseases. One of the possible links are enhanced pro-inflammatory immune responses, which might be due to an increased permeability of the epithelial barrier. Probiotics may decrease the intestinal permeability, and therefore help to reduce the frequency and/or intensity of migraine attacks. The main aim of this study was to investigate the influence of daily intake of probiotics on the frequency and intensity of migraine attacks among migraine patients.

During this open-label pilot study of twelve weeks, the participants (n=27) took 2 grams of a probiotic food supplement (EcologicBarrier, 2.5x109 cfu/gram) per day. Participants kept a headache diary and filled in two validated headache questionnaires at baseline and after completion of the study. They visited the research centre every four weeks. To measure compliance, the participants were asked to take the probiotics that they did not use to the next appointment.

The baseline questionnaire showed that the mean number of headache days per month was 6.7 (SD=2.4). This number decreased significantly in week 5–8 of probiotic use to 5.1 (SD=2.2) and in week 9–12 to 5.2 (SD=2.4). The intensity of migraine also decreased significantly; the baseline score of a validated questionnaire was 6.3 (SD=1.5) on a scale of 0–10, and after the intervention period the score was 5.5 (SD=1.9). No significant differences between participants with and without gastrointestinal complaints were found.

To our knowledge, this is the first study which investigated the effects of probiotics on migraine. We found a decrease in migraine frequency of 23% and in severity of 13%. As this was an open-label trial, placebo effects cannot be ruled out. However the results are promising and warrants further controlled interventions which include measurement of intestinal permeability.

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Intestinal Dysbiosis in Overweight/Obese Subjects: Utility of Breath Test

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Hydrogen Breath Test (BTH2), based on a lack of source for hydrogen gas in humans other than bacterial metabolism of carbohydrates, is a useful and simple tool to detect the Small Intestinal Bacterial Overgrowth (SIBO) by evaluating carbohydrates malabsorption.

Obese subjects with/without Metabolic Syndrome (MS) often suffer from gastrointestinal disorders and intestinal dysbiosis; up to now, few data are available on these subjects.

In order to evaluate the clinical-diagnostic utility of BTH2, one hundred and six consecutive subjects (38 overweight, 68 obese; 16 males/ 90 females, aged 46.8±13.8, mean BMI: 33.2±5.6 Kg/m^2) were recruited at the "Obesity and Work" outpatients clinic of the Clinica del Lavoro "L. Devoto" Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico (Milan, Italy).

All of them provided written informed consent prior to participating in the study, completed a medical history and a physical activity/well-being questionnaire and were asked to follow a dietary plan for two weeks before BTH2. At least one day before the test, subjects were instructed to take a low fibre diet.

BTH2 was carried out both after overnight fasting (T0) and after Oral Glucose Tolerance Test (OGTT; T1). Hydrogen gas is generally detected in air you exhail and its cut-off value was settled at 12 ppm.

At T0 BTH2 of 5% of overweight subjects and 37% of obese subjects was positive.

Particularly, BTH2 was positive in 46% of subjects with MS and in 30% of those without MS.

After OGTT, 50% of BTH2 results, negative at T0, became positive.

Obese subjects with MS had an H2 altered value at T0 suggesting a intestinal dysbiosis.

On the basis of our preliminary data on obese-overweight subjects, BTH2 at T0 can be helpful to diagnose gastrointestinal disorders, while at T1 BTH2 positive results seems to be overestimated since it has lower specificity.

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Post-Prandial Incorporation of Marine Omega-3 Fatty Acids into Plasma Triacylglycerol and Non-Esterified Fatty Acids When Consumed in Different Chemical Forms

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Study Aim: To investigate whether different chemical forms of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (triacylglycerol (TG), ethyl ester (EE), free fatty acids (FFA)) are equally available and incorporated into human plasma triacylglycerol and non-esterified fatty acid pools in the post-prandial period.

Background: EPA and DHA may be found in seafood and in supplements in different structural forms. From all of the available literature, it is not clear as to whether EPA and DHA are equally available to the human body when presented in these different chemical forms.

Study Design: Healthy male volunteers (n 10) aged 18–40 years were recruited into a double blind cross-over trial. Each volunteer consumed EPA and DHA in capsules, and in different chemical forms (TG, FFA and EE); all volunteers consumed all chemical forms in a predetermined random order. All supplements used had the same ratio and amount of EPA and DHA (1.8 g). Volunteers were cannulated in the fasting state and blood was collected at baseline; volunteers then consumed a standard breakfast and the capsules and blood was collected at 9 time points over 6 hours. Plasma was isolated at all time points. All 10 volunteers took part in all post-prandial study days, which were at least 14 days apart. Incorporation of EPA and DHA into plasma triacylglycerol and NEFA fractions was measured using gas chromatography.

Results: There were no significant differences between chemical forms with regard to the appearance of EPA and DHA into plasma triacylglyerol or non-esterified fatty acids over the 6 hour post-prandial period.

Conclusions: From this data, EPA and DHA presented to the human body in different chemical forms appear to be equally bioavailable. The phospholipid form was not examined.

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