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LMC International Food Congress 2006: Nutrigenomics and Health – From Vision to Food

The Royal Veterinary and Agricultural University, Denmark,

15–16 March 2006

Organized by the Centre for Advanced Food Studies (LMC), The Royal Veterinary and Agricultural University, Rolighedsvej 30, 1, 1958 Frederiksberg C, Denmark

Preface

Welcome to LMC International Food Congress 2006: Nutrigenomics and Health – From Vision to Food

The Centre for Advanced Food Studies (LMC) is pleased to welcome you to the LMC International Food Congress 2006 at the Royal Veterinary and Agricultural University (KVL) in Denmark on 15–16 March 2006.

The aim of the congress is to disseminate the latest research, innovations and visions within this new and burgeoning field nutrigenomics.

The congress will bring together scientists and representatives from industry from all over the world, to meet, network and discuss current ideas and possibilities for the future, thus contributing to the enhancement of the international collaboration in nutrigenomics.

The programme of the congress will include invited keynote speakers, invited opening speakers, short oral presentations and finally a number of posters presented at the poster session.

Research in the field of nutrigenomics is multidisciplinary and demands the involvement of several disciplines. Presentations on the first day of the congress will explore topics in the field of "Nutrigenomics, markers and health". The programme on the second day will address topics in the field of "Nutrigenomics and new food".

The plenary sessions will comprise keynote lectures given by internationally respected scientists, who have been invited to present current and future research. Topics to be addressed will include nutrigenomics and the obesity problem, bioinformatics, the industry's expectations for nutrigenomics in relation to consumer health, system biology in Chinese nutrition and medicine, visions for nutrigenomics and pertinent ethical aspects.

The parallel sessions comprise in-depth lectures given by international experts on current topics of nutrigenomics. Each session will be opened by a well-reputed scientist of high international status. The short lectures in the parallel sessions will be based on selected submitted abstracts on the topics of "Biomarkers and prevention of lifestyle-related diseases", "Animal models in human nutrition research", "Civilization, ethics and marketing", "Gut, health and immunity", "Food for obesity prevention" and "Future foods for health".

Complementing the plenary and parallel sessions, a poster session entitled "Food and health" will give more scientists the opportunity to present their data, giving the congress delegates further opportunity to learn about and explore the many aspects of nutrigenomics.

This issue contains some of the papers constituting the basis for the key lectures and opening lectures, the selected abstracts for the parallel sessions and some of the abstracts for the poster session.

Director Lisbeth Munksgaard
Centre for Advanced Food Studies, LMC

Contents

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Programme overview

Wednesday March 15, 2006: Nutrigenomics, markers and health

08:15–09:00 Welcome and registration
09:00–09:15 Official opening
09:15–09:30 Welcome and introduction
09:30–12:55 Plenary session 1
12:55–13:55 Lunch
13:55–16:00 Poster session: Food and health
16:00–18:10 Parallel sessions:
1. Biomarkers and prevention of lifestyle-related diseases
2. Animal models in human nutrition research
3. Civilization, ethics and marketing

19:15–23:00 Congress dinner at Restaurant Moltkes Palæ including poster award

Thursday March 16, 2006: Nutrigenomics and new food

09:00–09:05 Welcome
09:05–09:40 Kick-start lecture
10:00–12:10 Parallel sessions:
4. Gut, health and immunity
5. Food for obesity prevention
6. Future food for health

12:10–13:10 Lunch
13:10–16:10 Plenary session 2
16:10–16:30 Coffee

Keynote speakers, 15–16 March 2006

Professor Wim Saris, Maastricht University, NL
Nutrigenomics and the obesity problem: hype, help or hope?
(*Short paper will be distributed at the congress*)

Dr Elaine Holmes, Imperial College, UK
Metabolite profiling strategies: Promoting a holistic vision of health and nutrition
(*Short paper will be distributed at the congress*)

Dr Ben Van Ommen, TNO Nutrition and Food Research, NL
From nutrition to nutrigenomics and back again-from technology push to mature science?
(*Short paper will be distributed at the congress*)

Chief Science Officer Peter Olesen, Chr. Hansen A/S, DK
Nutrigenomics and health – a new knowledge and innovation platform to meet user expectations: Industry, authorities and consumers
(*Short paper will be distributed at the congress*)

Professor Adrian Tsang, Concordia University, CA
Genome-wide approach to identify and characterize fungal extracellular enzymes
Kick-start lecture

Professor J. Bruce German, University of California, USA
Metabolomics as health assesment: Bringing nutrigenomics to practice

Professor Terry Graham, University of Guelph, CA
The impact of grains and breads on human health: The need for interdisciplinary research

Professor Arne Astrup, The Royal Veterinary and Agricultural University, DK
Prevention of obesity – foods that promote a negative energy balance based on bioactive ingredients

Professor Peter Sandøe, The Royal Veterinary and Agricultural University, DK
Ethical perspectives on nutrigenomics and novel foods
(*Short paper will be distributed at the congress*)

Associate Professor Jennifer Wan, The University of Hong Kong, UK
Nutrigenomics: Exploiting system biology in Chinese nutrition and medicine

Genome-wide approach to identify and characterize fungal extracellular enzymes

Adrian Tsang*, Natalia Semova*, Kathleen Daigneault*, Nicholas O'Toole*, Regis-Olivier Benech*, Gregory Butler*, Reginald Storms*, Justin Powlowski*, Thibaut Wenzel**, Rutger van Rooijec**, Anja Riemens** and Rob Meima**

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Introduction

Enzymes are protein catalysts that perform a wide range of chemical reactions on every conceivable natural material under a variety of environmental conditions. They drive the metabolic activities of microbes that have been used for thousands of years in the production of food and alcohol, and are commonly-used food additives today. Enzymes are specific in their action and can be used efficiently and safely to modify fats, carbohydrates and proteins in the production of specialized foods that improve health (1). Therefore, enzymes are expected to play an important role in the development of personalized nutrition, which is one of the main objectives of nutrigenomics.

All organisms make enzymes which support their lifestyles. Most fungi adopt a nutritional strategy in which they secrete extracellular enzymes to break down complex substrates and then transport the resulting nutrients into the cells for consumption. To accommodate this way of life, fungi have evolved effective, diverse and comprehensive arrays of catalytic activities. Fungal species, including *Aspergillus niger*, *A. oryzae*, *A. aculeatus*, *A. japonicus*, *Rizopus oryzae*, *Trichoderma reesei*, *Talaromyces emersonii*, *Kluveromyces marxianus* and *Saccharomyces cerevisiae*, have a long history of use in the food industry and are assigned a generally recognized as safe (GRAS) status by the United States Food and Drug Administration.

Extracellular enzymes from many fungi have already found uses in food applications and the search is on to find new and improved enzymes for food processing. To facilitate the search for new enzymes, we report here the use of a functional genomic approach to identify fungal genes that encode extracellular proteins. The expression of these genes in heterologous hosts and the biochemical characterization of the expressed proteins will be discussed.

Objectives

The long term goal of the project is to identify and produce enzymes with new functionalities, and use them in environmental and industrial applications including food modifications. In the current phase of the project, we are focusing on the identification and characterization of extracellular enzymes of fifteen fungal species.

Methodology

Construction and normalization of cDNA libraries

The fungi were cultured under different conditions. Total RNA was extracted using TRIzol® reagent (Invitrogen, Burlington, ON). Approximately 200 μg of total RNA from each culture condition were pooled and poly(A)+ RNA was purified by oligo-dT cellulose column chromatography (Amersham Biosciences Corp, Piscataway, NJ). Analysis of RNA integrity and quantification was performed by running RNA samples on an Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA). The cDNA library was constructed using a Zap-cDNA® Synthesis Kit and directionally cloned into the pBluescript® KS + vector (Stratagene, La Jolla, CA). The cDNA libraries were normalized by the negative selection method (2).

Sequence analysis

Plasmid DNA from the cDNA clones was prepared by alkaline lysis and sequenced from the 5'end using ABI 3730 XL automated sequencers (Applied Biosystems, Foster City, CA) at the Genome Quebec Innovation Centre. The chromatograms obtained following single pass sequencing of the cDNA clones were processed using Phred (3,4) to assign sequence quality values, Lucy (5) to remove vector and low quality sequences, and Phrap (http://www.phrap.org/) to assemble overlapping sequences derived from the same gene into contigs. The protein-coding regions of the sequences were predicted using OrfPredictor (6). Sequence similarity searches against the NCBI non-redundant database were performed with BLASTX (7). TargetIdentifier (8) was used to assess if the cDNA clones contain intact open-reading frames and to assign putative function.

Proteins targeted to the extracellular space by the classical secretory pathway possess an N-terminal signal peptide, composed of a central hydrophobic core surrounded by N- and C- terminal hydrophilic regions. We used Phobius (9) to recognize the presence of signal peptides encoded by the cDNA clones. The tools TargetP (10) and Big-PI Fungal Predictor (11) were used to remove sequences that encode proteins which are targeted to the mitochondria or bound to the cell wall. Finally, sequences predicted to encode soluble secreted protein by these automated tools were analyzed manually.

Cloning and expression of target genes

The protein-coding regions of the cDNAs encoding secreted proteins were PCR amplified using gene-specific primers that contain the Gateway (Invitrogen, Burlington ON) recombination sites. The PCR products were cloned into the Gateway donor vector and then to destination vectors of Aspergillus niger and Pichia pastoris (unpublished data) by recombination. The resulting clones were transformed into A. niger and P. pastoris for expression.

Enzyme characterization

We used assays in microplate and multiwell agar-plate formats to screen the expressed proteins for activity on over twenty substrates. These substrates are capable of detecting a wide range of activities including lipolytic, proteolytic and carbohydrate-degrading enzymes activities. Using appropriate substrates, these enzymes were then subjected to basic characterization including pH and temperature optima, and pH and temperature stability.

Results and discussion

Expressed Sequence Tags (ESTs) of fungal genes

We have used the ESTs approach to identify genes in the following fifteen fungal species: Amorphotheca resinae, Aspergillus niger, Aureobasidium pullulans, Coprinus cinereus, Cryptococcus laurentii, Cunnignhamella elegans, Geomyces pannorum, Gloeophyllum trabeum, Lentinula edodes, Leucosporidium scottii, Ophiostoma piliferum, Phanerochaete chrysosporium, Sporotrichum thermophile, Thermomyces lanuginosa, Trametes versicolor. This evolutionarily diverse collection includes three white-rot fungi, one brown-rot fungus, two thermophilic composters and two freeze-tolerant composters. In all, we have performed close to 200 000 sequencing reactions and the sequence reads assemble to over 71 000 unique sequences. We have obtained 4000–6000 unique sequences per species. The genome of each of these species is estimated to harbour about 10 000 genes. Hence, our effort to date has identified approximately half of the genes estimated to be present in these fifteen species. The annotated sequences can be obtained via the web site https://fungalgenomics.concordia.ca. We have compared the sequences between fungal species from this project and those available publicly. The ascomycetes and basidiomycetes share in common less than 50% of their genes. Among related species, for example between Aspergillus niger and Aspergillus nidulans, about 20% of the genes are unique to each of these species. Based on this initial analysis, the fungi examined so far possess a vast pool of diverse genes.

Soluble secreted proteins of fungi

Following automated and manual annotation, we determined that 3473 genes out of the 71 000 unique sequences are encoding soluble secreted proteins. Based on sequence comparison to known genes, we can assign putative function to 1682 of the genes predicted to encode soluble secreted proteins. For the remaining 1792 genes, we cannot discern their function based on sequence analysis. Over 95% of the genes with putatively assigned function are predicted to encode enzymes. The most frequently found genes are those encoding proteases, followed by endoglucanses, hemicellulases, lipases, cellobiose hydrolases, amylases, and beta-glucosidases. Since almost all of the genes with assigned function encode hydrolytic enzymes, it can be argued that most of the genes encoding secreted proteins of unknown function are enzyme-encoding genes. The large number of unique genes with unknown function possessed by these species may be a reflection of their diverse lifestyle. These results also point to the high probability of identifying truly novel enzymes, some of which may catalyze defined substrates that are useful in food processing.

Heterologous expression of fungal genes

In the first phase of analysis, we transformed the same set of genes encoding secreted proteins with predicted function into the filamentous fungus Aspergillus niger and the yeast Pichia pastoris to express recombinant proteins. About 40% of this selected set of well-annotated genes of interest can be expressed in A. niger functionally. Most of the genes that can be expressed in A. niger can also be expressed in P. pastoris. Furthermore, most of the genes that cannot be expressed in A. niger are also not produced in P. pastoris. Expression in A. niger was induced by fermentation in maltose while expression in P. pastoris was induced by methanol. The two hosts and the two culturing conditions are very different, and yet they display similar preference in the expression of heterologous genes. Further

analyses, including examining the integrity of the original clones, are required before we can determine why some genes are more difficult to express than others. Since proteins are expressed at higher levels in A. niger than in P. pastoris, we are now focusing our expression studies in A. niger.

Analysis of the expressed proteins

The heterologous proteins are harvested from the culture medium of the expression hosts and removed from the medium using size-exclusion membranes. Basic biochemical characterizations such as temperature optimum, pH optimum and temperature stability are performed on the recombinant proteins using defined substrates. One of the aims of the project is to establish a library of enzymes with a wide spectrum of functionalities. The results obtained so far show that the enzymes in our collection are active in a wide range of temperature and pH. Based on the enzymatic activities characterized, we can predict that they are potentially useful in many different industrial applications. Testing a large number of enzymes for utility in a wide range of industrial applications poses logistic challenges that are beyond the scope of an academic laboratory. Our research collaborators at DSM Food Specialties of the Netherlands are developing assays to test the usefulness of these enzymes in food applications.

Conclusions

Enzymes are specific and efficient in modifying foods, and they are expected to play an important role in the production of specialized foods with health benefits. Genomic approaches such as the one described here provide rapid means to identify and characterize enzymes with different functionalities. The function of most secreted proteins of fungi cannot be predicted using currently available bioinformatic tools. Many of the proteins are expected to represent new enzymes.

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Metabolomics as health assessment: bringing nutrigenomics to practice

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Introduction

The 21st century is facing a major threat to human health and to the value of the industrial enterprises of food and agriculture. An increasing number of individuals in populations around the globe are suffering diseases that are caused by poor diets. Paradoxically, these are not the same diet-related diseases of nutrient deficiencies that were epidemic before the 20th century; but instead they are the result of the routine consumption of diets that are so unbalanced as to dysregulate normal metabolism. It has been recognized for decades that health—as metabolic regulation—is responsive to various aspects of the environment, including diet and exercise. It has also been recognized that although common patterns of diet and health exist among populations, some individuals are responsive to a particular environmental change and express significant changes in health outcome as a result and some individuals are conspicuously non-responsive. The modern trend in obesity is just such an example. Fully two thirds of all adults in the United States will spend at least a portion of their lives overweight, and half of those will be obese. Because this percentage has more than doubled in the past 30 years, it is considered to result from a purely environmental change, i.e., non-genetic causation. Nonetheless, one third of the population in the same environment is completely free of overweight and obesity; therefore, genetic predisposition to disease is considered to be as important as environment. Furthermore, it cannot be said that the fraction of the population that is

apparently genetically protected from obesity is simply inordinately healthy. This is because accurate assessment within the entire population shows that predispositions to allergies, intolerances, increased susceptibility to infectious disease, reduced response to immunizations and many other environmentally-dependent diseases are also distributed widely, but quite differently than obesity. Public health is thus faced with two monumental challenges—the diseases that are becoming epidemic are not caused by a single factor and cannot be resolved by a single curative solution; all individuals in the same environment do not respond identically to diet, thus the same dietary advice will not work for everyone.

There are different possible solutions to the types of health problems that are the result of a combination of genetic predisposition and environmental stimulus. First would be to simply invest in diagnostics and curative therapeutics, wait until the disease phenotype is expressed in an individual and can be diagnosed, and then and only then, attempt to reverse the disease in those expressing it. Second would be to invest in understanding the genetics of individual predisposition to environmentally-induced diseases and bring this genetic knowledge to practice as a form of genotyping diagnostics in which recognizing an individual's predisposition to diseases is a part of routine medical surveillance. Third would be to invest in explicitly measuring and understanding the basis of variations in metabolic phenotype as the means to assess health, distinguish metabolic differences within individuals that represent trajectories towards adverse health outcomes, and intervene with diet and lifestyle modifications to change their metabolic phenotypes before they develop into disease. The first option assumes that metabolic diseases and their damage are reversible, which they are not. The second option assumes that the mechanistic implications of sequence variations in the human genome can be understood to the point of accurately predicting health, which will not be true for some time into the future. Therefore, we are left with the third option of developing high-throughput analyses of metabolites and our understanding of metabolic variation to the state of development that they can comprehensively and quantitatively measure metabolism in an individual with sufficient accuracy to be predictive of present and future health.

Steps necessary to bring metabolic profiling to practice

Ontology of metabolic health

Key to the success of genomics has been the coordination of global scientific knowledge of genes leading to a process of concerted annotation and to structured vocabularies like the Gene Ontology. This consolidation of information into concentrated, publically accessible knowledge bases is not just accelerating research but actually leading to synergistic research strategies. For example, bioinformaticians are increasingly able to predict unknown functions of genes by comparing their sequences to similar sequences whose expression profiles have already been annotated with functional/structural information. Similarly, metabolic pathways are increasingly being described by annotating genes as enzymes—including information about their reactions, allosteric regulators, affinities to substrates, their locations within different organisms, tissues and cells—and orienting these genes and pathways within the knowledge of overall biochemical metabolism among disparate organisms and their evolutionary relationships.

Bioinformatic successes in genomics will need to be translated to a similar and interconnected knowledge base of metabolites. A common, publically accessible metabolomics knowledge base will guide scientific researchers the same way that genomics ontologies are guiding all of life sciences. Just as functional genomics is discovering which genes are related to which gene families, metabolomics must discover not only which metabolites are derived from which other metabolites—and how—but which metabolites interconnect different metabolic pathways, which metabolites clearly limit pathway fluxes and which regulate overall systemic control. Because diet is so critical to metabolism and the abundance, flux and ultimate disposition of metabolites, diet will become a critical "annotation" parameter to metabolite ontologies. As other aspects of the overall organism's environment are also important to metabolism, such databases will eventually need to include key environmental parameters as well. Importantly, those investigators/fields representing the various aspects of environmental influence to health, including diet, will need to develop quantitative and absolute variables to these annotation processes. Needless to say, more accurate measures of caloric and nutrient intakes will be needed; for example, the phrase "high fat diet" is insufficiently precise to become a predictive annotation variable in a metabolome ontology.

The advances in genomics are increasing our knowledge about genes and the structures/functions/systems to which they give rise. Knowledge is often sufficient to predict the outcome of the lack or failure of genes, i.e., genetic diseases. Unfortunately, genomics information does not as yet provide sufficient quantitative relationships to predict a system's overall success, i.e., health. The Human Genome Initiative has, in fact, recognized this need and established as one of its Grand Challenges: "II-2, Develop strategies to identify gene variants that contribute to good health and resistance to disease" (Guttmacher and Collins, 2005, Collins et al., 2003). The same challenge is equally vital to the future of metabolic assessment. It is necessary to build knowledge about individual metabolites—*and* ensembles of metabolites—and ultimately about how they interact to impact health/nutritional status. As a parallel to genomics, the goal is to identify metabolic states that contribute to good health and resistance to disease. To date, information about the neither the relationship between genes and health nor metabolites and health has been codified into formalized ontologies or databases. Proof-of-principle for annotation about the failure of health already exists, but it is based on disease and annotations of SNPs, nutrient deficiencies and/or exposure to toxins that are the basis of disease. This approach to defining diseases has led to the emergence of biomarkers of disease and the commercialization of the approach as diagnostics built on the clinical measurement of these biomarkers. Unfortunately, health and disease are not

the same. Annotating genes, their products, nutrients and/or metabolites with a more generalized perspective on health, particularly as its success, has not been established. One of the challenges in developing "health" ontologies as opposed to "disease" ontologies is that health is currently ambiguous, abstract and poorly defined. What is needed is a deconstruction of health as a detailed definition, its components, their breadth of normal variation, causal relationships between variation in health and protection from disease, and a suggested framework for the development of health/nutrition ontologies. Widespread adoption of such "health" ontologies will provide a formalized conduit through which newly discovered biological knowledge can be translated into individualized, evidence-based clinical practice.

In addition and in parallel to building structured ontologies of health, it will be necessary to build the detailed supporting scientific evidence as structured reservoirs of data from precise phenotypic measurements of health. Metabolomics as a field has a unique opportunity to construct a formalized knowledge repository structure prior to the acquisition of the metabolic data that will fill it. The human genomics initiative has taught the scientific community many valuable lessons about the pursuit of large biological databases with shared resources and disparate goals.

Establishing the human metabolomes

Before it is possible to assign normality to metabolism, it is necessary to establish in precise molecular terms what are the human metabolites and what are their ranges within the "normal" population. This issue has created a great deal of controversy as different scientists approach the problem from their specific analytical, physiological, biochemical and genetic backgrounds. First, it must be agreed that in contrast to a human genome that is relatively well defined, constant in an individual and presently technologically measurable, the concept of a metabolome is in many ways undefined, spatially and temporally dynamic, and technologically not measurable. There is no single metabolome, but instead an infinite number of possible samples. Therefore, it is necessary to define a set of conditions in which a cell, tissue or biofluid could serve as a defined sample and the metabolites resident within it measured. For humans—in addition to isolated cells and tissue samples—blood, urine, saliva, tears, cerebrospinal fluid and lymph have been proposed as biologically-relevant samples for metabolomic analyses. For nutrition—because of its role in providing a reservoir of nourishing substances, substrates, intermediates and metabolic products—blood is at present the most logical candidate to be a first priority metabolomic biofluid.

Once blood, for example, is defined as a consensus sample, two discrete approaches could be taken to defining the "metabolome." One approach would be to define from biochemical terms what are the endogenous metabolites in humans—i.e., all of the intermediates in all of the necessary biochemical pathways—and establish this specific set of metabolites as the human metabolome and then assemble the analytical tools to measure each of these defined compounds in a sample of blood. A second approach would be to measure all small molecules within a sample of blood and define them as the constituents of the human metabolome. While these seem like simple alternatives, proponents of each approach are enjoined in a heated debate as to what is and is not human metabolomics. There is a very practical answer with respect to nutrition and nutrigenomics. If the latter approach were adopted, it would take literally decades to assemble the analytical tools to measure all of the molecules that could be found in human blood. If the former approach of defining all of the endogenous metabolites necessary to human life were defined, the list could be defined and the tools to measure them assembled within months. For nutritional health in which it will be critical to begin to assemble databases of the ranges of human metabolites as a function of dietary, lifestyle and genetic variations, the second approach would appear to be the most logical.

Scope of the endogenous human metabolome

Estimates of the total number of human metabolites that constitute the endogenous metabolome could be obtained by examining basic metabolic pathways, measuring metabolites from cells and tissues in vitro, and measuring the metabolites within germ-free animal models fed purified diets. The total number of metabolites in humans that are endogenous and necessary to life—and restricted to monomeric units of biomolecules rather than oligomers of peptides, sugars, nucleotides and lipids, is estimated to be approximately 3,000. This is much smaller than the number of metabolites obtained by including contributions from endogenous microflora, potential secondary plant metabolites arising by consumption of plant-based foods, and the myriad metabolic products that result from the reactions between secondary plant metabolites and the combined actions of human and microbial metabolism. Combining all of these inputs, the total number of small molecules that could be measured in human blood could easily exceed 50,000. The decision to choose the smaller, endogenous pool is not simply pragmatic, however. From a health perspective, the importance of an exogenous small molecule is typically that it can influence endogenous metabolism. Hence, if all—but only all—endogenous metabolites were measured accurately, even if it was not possible to immediately assign the molecular cause of a variation in metabolism caused by an exogenous agent, it would be possible to detect its influence. Furthermore, if an exogenous metabolite did not cause a change in endogenous metabolism, could it be considered of biological, or at least nutritional, importance? Once the approximately 3,000 endogenous metabolites were defined, the critical role in establishing the human metabolome would be to define the quantitative abundance and biological range of these metabolites. Fortunately, for many metabolites, this process has been pursued for decades, and some very practical and conceptual lessons can be learned. Cholesterol levels have been measured on an absolute scale in human plasma for decades. The mean concentration of cholesterol in human adults is approximately 200 mg/dl, and the range about the mean has a standard deviation of approximately 25. Thus, in moving to a high-throughput instrumentation

platform, the analytical specifications for cholesterol as one of the 3,000 metabolites are known. To date, such knowledge exists for perhaps 1,000 of the endogenous metabolites in blood. The challenge is to complete this database and, on this foundation, begin the process of annotating the molecular, mechanistic causes and phenotypic consequences of variations in the absolute concentrations of the endogenous metabolites.

Annotating the human metabolome

The opportunity available to the field of nutrition as integrated metabolism is to assemble sufficient understanding of overall metabolism and its regulation by diet to provide the knowledge resource necessary to a new industry of clinical health management. The challenge is to assemble a quantitative, comprehensive understanding of the breadth of metabolism once the tools are in place to measure it. Meeting such a challenge will require both a focused understanding of specific metabolites and also a comprehension of metabolism as an integrated whole. Once again, lessons from the field of genomics have proven to be insightful for metabolomics as well. Annotating the human genome is now the goal of functional genomics and while it is true that single, unknown genes are being identified on a daily basis, rapid progress also is being made in understanding the functional clustering of genes as gene sets, and how these various gene sets interact. Several studies have documented that the clustering of genes can be identified using gene expression arrays, for example, the coordinated regulation of genes associated with cholesterol metabolism (Horton et al., 2003). Results from dietary lipids have previously shown that dietary factors can similarly act through clearly coordinated gene sets (Mutch et al., 2005). The basic approach of clustering genes according to biologically supervised criteria has now even been brought to high-throughput bioinformatics (Subramanian et al., 2005). In essence, biological genomes are being assembled into their biological hierarchical structures. Once these gene sets are recognized as discrete clusters, the power of gene expression analysis will increase dramatically. First and fore most, the statistical power of gene expression technologies (arrays, beads, etc.) is insufficient to provide confidence of a single gene event in any but a subset of highly expressed and also highly responsive genes. However, by assembling multiple functionally-related genes as clusters, the process of interpreting gene expression becomes a biologically supervised and powerful investigative tool (Mootha et al., 2003). Further, as it becomes possible to identify more than independent gene regulation events, the coordinated regulation of cellular functions begins to emerge. Not surprisingly, simultaneously with the recognition that gene sets are coordinately regulated, the mechanisms of this regulation are emerging as well. Transcription co-activator proteins have emerged whose sole function appears to be to participate in the sensing of cellular signals and transducing these signals into the recruitment and modification of transcription regulation by classic transcription factors (Lin et al., 2005).

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The impact of grains and breads on human health: the need for interdisciplinary research

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Introduction

The mandate for this conference is to "present current and future research and ideas such as nutrigenomics and the obesity problem". Obesity is associated with a high risk for type 2 diabetes and both of these ill-health problems are strongly associated with cardiovascular disease (the ill-health triad). Clearly, the triad is of vital importance not only to the health of the Western world but to also that in developing nations. Health care in the Western world has focused on the treatment of the diseases and paid little attention to that of primary (preventive) health care. Society has benefited greatly from this, but it comes at a large economic cost and, all too often, without a marked improvement in the quality of

life. Willett (1) pointed out that the treatment of disease is extremely expensive, using as an example the pharmacological treatment of high cholesterol with statins. This could cost USA $30 billion dollars, would result in a modest lowering of the incidence of coronary heart disease and does not address the underlying causes of the ill-health. Lifestyle changes in nutrition and activity can have a great impact on preventing or delaying the triad. This report will highlight the author's views on the requirements for present and future research in this area by identifying elements of the assimilation of carbohydrates in breads as an example of the complexity of the problem, and then by paying some attention to polymorphisms and gene expression.

The complexity of foods and their assimilation

Health status is often monitored by the assessment of traditional biomarkers (e.g. blood lipid profile and glucose) following an 8–12 h fast. However, humans spend at least 18 h per day assimilating nutrients (2, 3) and this complex process is associated with large changes in many biomarkers that reflect the risk for type 2 diabetes and cardiovascular disease (2). Recently, it was reported (4) that the postprandial blood glucose concentration is an independent risk factor for cardiovascular disease in type 2 diabetics (especially in women) and is superior to fasting glucose in predicting risk. There is no doubt that the metabolic changes associated with assimilation of nutrients are complex and can be associated with negative changes in biomarkers.

Canadians are recommended to consume five to 12 servings of grain products a day and the average person aged 35–65 years barely achieves the minimum recommendation (5). Breads represent 40% of all carbohydrates consumed in Canada and are excellent grain products. Breads can have remarkably different properties based on not only the type of grains used, but also how they are milled, the combination of ingredients, the leavening process and the baking conditions. The amount of resistant starch, fibre, organic acids, etc., can influence gastric emptying and the ease with which the starch can be hydrolysed to monosaccharides. The stomach and gut produce a wide array of powerful hormones that influence the assimilation process as well as complex sensations such as satiety. Among the critical hormonal signals are the incretins (glucose-dependent insulintropic polypeptide and glucagon-like peptide-1), which are responsible for a significant portion of the resulting insulin secretion. The assimilation process involves not only the liver and pancreas, but also muscle and adipose tissue. The former is responsible for the majority of the carbohydrate storage and oxidation, while the latter is responsible for changing levels of cytokines (adipokines) and related compounds such as tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), plasminogen activator inhibitor-1 (PAI-1) and C-reactive protein (CRP). These are now recognized as vital biomarkers of health. They can alter insulin sensitively/resistance and result in metabolic stress of the vascular endothelium. Even this very brief overview demonstrates that assimilation is a dynamic process. Research has shown that the degree of these responses is an important component of the metabolic syndrome and an early indication of a person's risk for type 2 diabetes and cardiovascular disease.

The complexity of the food science of breads provides diverse opportunities to develop healthier foods that could result in less disturbance in the homoeostasis of the biomarkers. For example, subjects in a study by Behall et al. (6) followed a diet of wholegrains with varying amounts of β-glucan. After 5 weeks, total and low-density lipoprotein cholesterol were reduced by as much as 10 and 17%, respectively. In another investigation (7), type 2 diabetics consumed a diet in which the types and sources of carbohydrates were not changed, but the structure of the starches was altered (e.g. parboiled rather than sticky rice; white durum pasta rather than white durum bread). After 24 days PAI-1 was reduced by 58%. In investigations of this nature, it is often noted that there is a large degree of intersubject variation in the degree of the responses. It is suspected that this may be due to the genetic complement of the individuals studied.

Nutrigenomics and the triad

The environment has a profound impact on gene expression and disease risk. People's environment is complex and includes many lifestyle factors, including smoking, alcohol consumption, exercise habits and diet. Nutrition is a unique factor as it impacts on every one of us and does so many times a day, whereas other components are not part of everyone's lifestyle. In addition, nutrition may offer more diversity than other lifestyle components in the impact on gene expression and health.

Many scientists have stated that although obesity appears to be a simple problem of a positive energy balance, the underlying mechanisms are very complex and involve hundreds of genes. It becomes even more complicated when one considers that common gene variants (polymorphisms) of many of these genes are also important in a person's relative risk for chronic disease states (8). Within the triad and metabolic syndrome, the genes involved include those for taste receptors, peripheral signaling peptides, central regulators of appetite and satiety, adipocyte growth, energy expenditure, substrate metabolism, traditional metabolic hormones, their receptors and signaling pathways. Considering this range of hundreds of genes and their polymorphisms, there will be no one "cure", no one function or only one tissue whose malfunction is the ultimate cause.

Loktionov (8) points out that attributable risks for a complex disease can sum to well over 100%, implying that the disease can be prevented in more than one way. In addition, polymorphisms typically have relative risk factors of less than 2 and are not strong enough to be easily identified. Therefore, screening for single genes is not likely to be very useful in identifying an individual as being at risk for the triad and metabolic syndrome. Families of genes are involved and single genes may only explain a part of one risk factor. Nevertheless, most individual investigations will need to focus on one gene or a few genes.

There have already been some interesting discoveries which, among other health benefits, may explain why some subjects/patients are more susceptible to the triad and/or less responsive to certain treatments. One aspect that has received attention is that of peroxisome proliferation-activated receptors (PPARs). The PPARs are members of the nuclear hormone receptor subfamily of ligand-dependent factors. One, PPAR-γ, has a number of genetic variants and is associated with many metabolic characteristics including adipocyte differentiation. Nicklas et al. (9) found that women who had the Pro12Ala variant of PPAR-γ were more susceptible to weight regain after weight loss. Moffett et al. (10) reported that another variant was associated with insulin resistance in women. Rhee et al. (11) recently reported that women who had two polymorphisms (Pro12Ala and T allele) of PPAR-γ simultaneously had greater weight and certain aspects of metabolic syndrome had a greater prevalence with specific alleles. It has also been found (12) recently that a combination of certain variants for both PPAR-γ and IL-6 on obesity related factors was additive. PPAR-γ coactivator-1α (PGC-1α), a nuclear coactivator, has been shown to coactivate the PPARs and to integrate various signaling pathways involved in metabolic gene activation and expression. Through coactivation of various transcription factors, PGC-1α is implicated in regulating the transcription of genes that encode proteins involved in glucose uptake and fatty acid oxidation. Mootha et al. (13) used a microarray system to examine 106 genes associated with oxidative phosphorylation in muscle from human diabetics. They found a modest (~20%) decreased expression of genes in 89% of these genes and this was associated with lower levels of PGC-1α.

While this area of study is in its infancy, even rarer are studies examining dietary perturbations and polymorphisms. Nevertheless, these show considerable promise. For example, in one study (14) subjects with various genotypes for IL-6 and TNF-α consumed fish oil supplements for 12 weeks. Irrespective of the body mass index, a combination of certain genotypes in adipokine genes was correlated with higher CRP and triglyceride concentrations and the degree of triglyceride-lowering by fish oils was influenced by the genotype for TNF-β. Byrne et al. (15) examined the PAI-1 postprandial response to lipemia associated with an oral fat tolerance test. The large intrasubject variation was accounted for by the presence of PAI-1 polymorphisms. Those with at least one 4G (deletion) allele had a much greater rise in PAI-1 and thus could be more susceptible to atherogenesis and thrombotic events.

This field of nutrigenomics is exciting, but we must also recognize that the answers will be complex. In addition, the term "nutrigenomics" is somewhat misleading. The concept is very accurate and important; the metabolic signals associated with assimilation of nutrients can alter gene expression and hence the capacity of the cell/tissue to perform its normal functions. However, it should be noted that the signals are those of metabolism and any other metabolic perturbation would have the same effect. For example, assimilation of a certain fat could result in a signal that alters PPAR-γ, but the same signal could also be induced by other metabolic perturbations such as exercise. The nucleus and the genes do not know the source of the signal. Without recommending that we introduce another new term (exer-omics?), there is a need to recognize that this field of study is "metabolomics". The reason for drawing attention to this point is to encourage those interested in nutrition and gene expression also to consider other fields of study (such as exercise physiology) in which metabolism is altered.

How to study such complex issues

When trying to gain an understanding of such a complex health problem it is difficult to know where to begin, how to conduct an investigation, how to develop a research programme. How does one begin to examine a research question such as why do some lean individuals develop metabolic syndrome (metabolically obese, normal weight people) and some obese individuals not develop metabolic syndrome (metabolically healthy obese people)? Science has become very reductionistic; some investigators refer to "their gene" and entire departments may focus on gene expression. These approaches to research are important and provide vital aspects to the understanding of topics such as metabolic syndrome and the triad. However, true and complete understanding of metabolic syndrome requires a great deal of integration. The problems require multidisciplinary approaches; not only many genes, but also many tissues of the body are involved. The inclusion of nutrition in the research means that an intimate understanding of food science is important, and this could be extended to horticulture and to genetics of crops and domestic animals. As we acquire an understanding of the physiology and nutritional metabolism, then it is critical to link to consumer studies and those who work in health policy. Knowing the basic science does not help if we cannot get the consumer to change their diet!

Clearly no one person and no one department can do all of this. The research must be focused and at least to a degree reductionistic. Just as we must ask how often we need to describe the problem in the intact human, we must also ask how we one reach an understanding by studying one gene, one transporter, one enzyme or one tissue. Thus, while there is a need to be reductionistic, there is an even greater requirement to be open minded, to work with others who are not in the same field, and to attend forums such as this one in order to gain an appreciation for other aspects of the scientific puzzle. This is the only way to see the entire solution.

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Prevention of obesity: foods that promote a negative energy balance based on bioactive ingredients

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Global epidemic

People are becoming more and more overweight, and this development is not limited to industrialized countries, such as North America and Europe, but is also seen in most countries in South America, the Middle East, Australasia including China, and parts of Africa. Even among the populations of some of the poorest countries in the world malnutrition and starvation exist side by side with overweight and obesity. In the light of this it is understandable that the World Health Organization has characterized the development as a global epidemic of obesity.

Serious impacts on health

In itself obesity could be considered to be primarily a cosmetic problem in a world where slenderness is the prevailing ideal body image, and for this reason obesity is accompanied by huge psychological problems leading to impaired quality of life, depression and the use of antidepressive drugs. However, from a medical point of view it is a far more serious problem that excess body fatness is the main driver of the epidemic of type 2 diabetes seen in all the countries where the prevalence of obesity is on the rise. Type 2 diabetes is a serious ailment and the diagnosis is synonymous with a 10 year reduction in life expectancy. This is due to an accelerated atherosclerosis with an increased occurrence of heart attacks and strokes.

Obesity is also complicated by heart disease, gallstones, various cancers, arthritis and infertility in both genders, not to mention all the health problems seen in overweight children.

How could things go so wrong?

The last couple of generations have been convinced that all technical and electronic innovations are a positive driving force for wealth, improved quality of life and comfort. However, we have probably failed to recognize the flipside of the coin; namely that the use of motorized transport instead of walking or cycling, and the dominance of our leisure time by computers, television and

video is associated with dramatically reduced muscular activity, with concomitant reduced caloric needs. More leisure-time physical activity, i.e. participation in sports, is clearly insufficient to compensate for the massive decrease in daily physical exertion.

At the same time our diet and foods have become much more fattening. This is not exclusively due to increased fat and sugar content in meals and soft drinks, but it is also caused by the increase in portion sizes, seen in everything from hamburgers and pizzas to chocolate bars and cola (1).

Are healthy foods boring?

Over the past 15 years the food industry has tried to cope with the first signs of the obesity problem through a single intervention, namely by reducing the fat content of foods. This has clearly proved insufficient, not least in compensating for the dramatically reduced levels of physical activity. More effort is now being made to reduce sugar content and increase fibre content, etc. All nutrition research suggests that the development of the obesity epidemic could be halted by improving dietary habits, i.e. by increasing the intake of less energy-dense foods, decreasing portion sizes and substituting water for calorie-rich beverages.

However, is it realistic to expect the population to comply with such dietary recommendations? As nutrition experts working in the field of public health we must admit that most of our attempts to induce the population to change their dietary habits over the past 20 years have not met with much success. Some achievements have been made, but we have been very unsuccessful in reducing caloric intake. Perhaps we have not really understood the real culprit?

The low-fat versions of many cuisines are patently bland and unexciting. This is particularly true for Scandinavian cuisine, which has been mainly based on butter, cream and full-fat cheese. When these high-fat ingredients are excluded from the diet the resulting dishes are more or less tasteless. However, much can be learned from many ethnic cuisines, such as Mediterranean, Thai, Mexican and Japanese cooking, where palatable foods are brought together with a sophisticated mixture of sweet, sour, bitter, salty and hot ingredients, resulting in special flavours. Many efforts are now focused on the fact that healthy foods should also be appetising and full of taste to satisfy the everyday need for gastronomic treats (2).

A challenge for the innovative food industry

Newer nutrition research has found that satiety or fullness, the major determinant of when we stop eating, can be manipulated simply through a better understanding of which components of foods affect the sophisticated signalling from the stomach and intestine to the brain. This cutting-edge research is now hot stuff for the food industry, which must translate the findings into more palatable foods that can be incorporated into the diet, and which will reduce the spontaneous food intake of the consumer, unconsciously, without dietary restrictions or connotations of asceticism. Among other properties of interest is the inhibition of fat absorption from the gut by naturally occurring substances in the food, which bind the fat so that it is excreted in the stools. Another area of interest is bioactive food ingredients that increase the body's caloric expenditure, helping to deal with excess calories in the diet and making it easier to maintain a normal body weight.

Natural foods that combat obesity

Natural foods with enhanced health-promoting properties are very attractive to the consumer, even though these may be more expensive. This is an extremely interesting area for product development for the food industry, fulfilling consumer demand and allowing a higher price for the product. But many issues remain.

Even though these ingredients occur naturally, high concentrations of these ingredients may be required to produce the desired biological effect. Is it safe for all individuals to consume higher quantities of these substances than they would normally be exposed to? A higher protein content was the secret behind the success of the Atkin's, South Beach and Zone diets (3). Other examples are calcium from dairy products that impair intestinal fat absorption and may produce weight loss (4). Other studies demonstrate that hot chilli, green tea and certain amino acids may increase 24 h energy expenditure in humans (5).

This poses safety questions and these perhaps demand clinical trials, such as are needed in the process of approval of new drugs in the pharmaceutical industry. Today it is known that industrially produced *trans* fatty acids in foods pose a major threat to human health (6) and such fats would today not have been given GRAS ("generally recognized as safe" (GRAS) status. Marketing and communication from the food industry to the consumer also pose some problems. Will the authorities allow health claims for specially developed food products that enhance satiety, reduce gastrointestinal caloric uptake and promote increased bodily heat production? These questions are not easily addressed, but they should not prohibit the development of the improvement of foods demanded by the overweight population.

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Linking Traditional Chinese Medicine to Systems Biology and Nutrigenomics

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Abstract

Dietary intervention to prevent, mitigate, or cure disease stretches back over 5000 years for the Chinese. Chinese Nutrition, follows the basic theories of Traditional Chinese Medicine (TCM), is presently taught as a multi-disciplinary system in China. This short article attempts to introduce the history, etiology, pathology and treatment principles of disease of TCM and linking its similar concept that "understanding human health depends on the holistic description of human biology and environmental cues that constant exposed" with the two recent emerged multi-disciplinary systems-systems biology and Nutrigenomics.

The Chinese believe that '*food and medicine originates from the same source*' and diet therapy is a common practice in daily life. Chinese nutrition, follows the basic theories of Traditional Chinese Medicine (TCM), is established around the 11th century –771 B.C. and presently taught as a multi-disciplinary system for the medical students in China (Zhang et al., 1990, Cai 1996). Chinese medicine views etiology, pathology and treatment of disease differently from western medicine. The occurrence and evolution of disease is believed to be a process of the loss of harmony between *yin* and *yang* - a philosophical concept of the dichotomous nature of everything in the universe, e.g. day/night, man/woman, excess/deficient, summer/winter, hot/cold (Liu 1998, Wu and Zhongbao 2000). *Yin* and *yang* represent opposing aspects within a single object and applies the concept to the human body, there is *yin* and *yang* at all levels: from the body as a whole, down to the level of molecules and genes., i.e. arteries (*yang*) and veins (*yin*); glucagons and testosterone are *yang*; insulin and estrogen are *yin*; RNA is *yang*, and DNA is *yin*. In all situations, they are interdependent and in opposing each other, create unity. A preponderance of *yin* and *yang* would result in disease and treatment principle is to restore their balance. The application of antagonistic or allopathic therapy is emphasized by using medicine and food with the opposite nature to that of the illness. For instance, for severe cases of hot diseases, 'cold' remedies are given, while for mild diseases, 'cool' remedies are indicated (Liu 1995, Wu 2000, Cai 1996).

In TCM and Chinese nutrition, herbs and foods are classified by their "basic nature", "flavor", and "effect" (Cai 1996, Zhang et al., 1990). Each food is considered to have a particular nature (cold, cool, warm and hot); a unique favor (sweet, sour, salty, bitter, or pungent), a specific organ/meridian target and dynamic force (i.e., cause energy to ascend, descend, to move inward or outward). Incorporating the *yin* and *yang* concept for classification, foods that are pungent, sweet, with ascending, floating and dispersing effects are *yang*; and foods that are sour, bitter or salty whose effects are descending, condensing and astringent are *yin*. Among the common foods, for examples, coriander, walnut, lobster, mutton, wine, vinegar, peach, onion, pumpkin are 'warm'; pepper, chili, ginger are "hot"; eggplant, soybean, lettuce, celery, apple, pear, tea, pear are "cool" and chrysanthemum, bitter melon, crab, salt, tomato, kelp, watermelon, banana, cucumber are "cold" in nature. Vegetables and fruits, with few exceptions, are cool and sour. According to TCM, eating chicken, pepper, dried ginger, (warm or hot nature), will worsen an illness of a "hot" nature (e.g. hypertension). For diseases of "cold" nature (e.g. winter flu, anemia, and hypoglycemia), the consumption of cold nature foods such as sugar cane, bitter melon, water chestnut, watermelon should be limited. Foods are also classified according to "favor". The five favors are sweet, sour, pungent, salty and bitter. Foods with sweet favor (e.g. milk, cream, mushroom, maize, soybean) are good for treatment of deficiency disease if to be consumed with limited amounts; sour foods (e.g. pears, vinegar, red bean, hawthorn, orange, tomato, grape) exhibit astringent action, help promote blood circulation and control excessive body fluid lost dues to diarrhea or excess sweating; bitter foods (e.g. bitter melon, wine, tea, liver) stimulate appetite and also antipyretic; and pungent foods (e.g. celery, ginger, onion, pepper, garlic) are capable of dispersing of vital energy thus often applied at the initial stage of the common cold. Each "favor" is associated with its own targeted organs/meridians. Foods with the sour, bitter, sweet and pungent favor are identified to be beneficial to the liver, heart; spleen and lungs, respectively.

Whether the *yin* and *yang* concept of disease and the biologic functions of foods/herbs describes by TCM will be ultimately explained by science awaits further investigation. Nonetheless, the "hot nature and pungent" principles of some species (Kawada et al., 1988, McNamara et al., 2005) can be attributed to their ability to activate the adrenal medulla to secrete catecholamine (regulates heat production and pain sensation). The antioxidant, hypolipidemic, and hypoglycermic evidence on tea (Cooper et al., 2005), and hawthorn (Rigelsky 2002) helps in explaining their heart-organ target specificity (i.e. prevention of heart disease). To give just one clinical example, garlic's health benefits have been elevated from folklore to clinical study. The anti-inflammatory mechanisms of garlic include suppression of TNF-alpha and interleukin-6 production, suppression of nitric oxide synthase (iNOS) activity, during bacterial infection (Makris et al., 2005).

Systems Biology and Nutrigenomics Share the Holistic Systems Description of Human Health by Traditional Chinese Medicine

The "holistic systems description" of human health concept recently discussed in the two newly merged multi-disciplinary systems, Nutrigenomics and Nutrigenomics (Muller and Kersten 2003, Desiere 2004, Ben van 2004, O'Malley and Dupre 2005), reflects the similar holistic or system biology approach thinking between modern science and TCM in the understanding human health. Few have debated on whether systems biology approach should be the most promising strategy in Nutrigenomics to determine the exact mechanisms of diet-related homeostatic control and whether nutrition should enter into the area of linking genetic differences with tailor-made nutrition, that is, given on an individual basis as "personalized nutrition" (Muller and Kersten 2003, Fenech 2005). The "holistic system description" of human health is the bases of TCM and Chinese nutrition (Wu and Zhongbao 2000, Liu 1988). The multi-targets treatment strategies in TCM not only aim to regulate several crucial pathological targets but, more importantly, the modulation of other associated general changes that delay the healing process (Jiang 2005). TCM encourages practitioners to use "personalized medicine" not only to accommodate the constitutional (genetic) make-up of patients but also able to adjust the climatic and seasonal conditions, and geographical localities (Zhang et al., 1990, Liu 1998, Cai 1996).

From the Chinese medicine research Conner, it is hope that knowledge of the sequencing of the human genome will help to better understand the *yin* and *yang* theory of the nutrition-health relationship, the adaptive capacity of genes and their functioning with the diet consumed. As the ear of molecular nutrition unfolds, a greater understanding on how circadian, climatic and season influence on health and disease describes by TCM will surely arise. It is now known that the human body is composed of millions of cellular clocks and oscillators whose co-ordinate activity gives rise to pronounced daily, monthly, and seasonal rhythms in physiology and behavior (Reppert 2001; Piggins 2002, review). These circadian clocks can function autonomously, independently of any external time cues, but can also be reset by environmental factors (Roenneberg et al., 2003) such as diet and light/darkness. Such understanding opens up enormous opportunities and questions, not at least of which is but whether every cell in our body has the potential to be circadian, how they communicate and how diet may affect the rhythms.

The questions need to be addressed in both Nutrigenomics and Nutrigenomics disciplines are: which are the diet-sensitive clock genes? Would seasonal foods versus purified and synthetic nutrient influence our clock genes differently and affect treatment outcomes? In cancer cells, circadian clocks control both cell cycle progression and apoptotic processes. The least toxicity and highest antitumor efficacy of the proapopotic drug docetaxel in bone marrow of mice was observed when dosing drug during the circadian rest phase (Granda et al., 2005). The protection on the bone marrow undergoing apoptotic cell death dictates by the balance between the cell cycle and apoptosis proteins Bcl2 and Bax (one good *yin* and *yang* example) is said to be under the control of the clock genes mPer2, mBmal1, mClock and mTim. Obviously, greater understanding of the cellular and molecular interactions of nutrients on our clock work is needed in systems biology and Nutrigenomics study.

In closing, I shall quote the saying by Jiang (2005) that "*in the struggle against diseases, we are lacking wisdom rather than knowledge*". That is true that scientific knowledge is important but yet, it is perhaps equally important to not lose sight of the value of wisdom.

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Opening speakers in parallel sessions, 15–16 March 2006

Parallel session 1: Biomarkers and prevention of lifestyle-related diseases

Professor John C. Mathers, University of Newcastle, UK
Prevention of lifestyle-related diseases

Parallel session 2: Animal models in human nutrition research

Professor Per T. Sangild and Professor Axel Kornerup Hansen, the Royal Veterinary and Agricultural University, DK
Animal models in human nutrition research

Parallel session 3: Civilization, ethics and marketing

Professor Ruth Chadwick, Lancaster University, UK
Statistical power personalised nutrition and ethics

Parallel session 4: Gut, health and immunity

Associate Professor Hanne Frøkiær, Technical University of Denmark, DK
Diet, genes and the immune system
(*Short paper will be distributed at the congress*)

Parallel session 5: Food for obesity prevention

Professor Richard D. Mattes, Purdue University, USA
Weight management through foods providing a metabolic advantage

Parallel session 6: Future food for health

Dr Jan Maat, Unilever Research & Development, NL
European technology platform "Food for life"
(*Short paper will be distributed at the congress*)

Prevention of lifestyle-related diseases

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Aetiology

Diet and other lifestyle factors play a significant role in the aetiology of all the major causes of morbidity and mortality including vascular disease, type 2 diabetes (T2D), osteoporosis, many cancers and dementia which burden humanity not only in economically developed countries but also in the urbanized areas of the developing world (1). The "health pendulum" in Fig. 1 attempts to conceptualize some of the key influences acting at various stages in the life-course that influence the risk of disease for a given individual (2). Although this simple model was developed for cardiovascular disease and some cancers, it is probable that it will be applicable for many common non-communicable diseases. For each individual, genotype determines the position of the fulcrum of the pendulum. That is, the pattern of genetic variations [largely encoded in single nucleotide polymorphisms (SNPs)] confers greater susceptibility or resistance to ill-health and determines, at conception, whether the bob of the pendulum is farther to the left or to the right. From then on, the pendulum can swing more towards the health or towards the disease end of the health–disease continuum depending on the relative strengths of the forces acting on the pendulum.

The initial promise that SNPs might be discovered that would explain individual susceptibility to complex (multifactorial) diseases has, in the main, not been realized. Many of the earlier reports of associations between specific SNPs and particular conditions, e.g. central adiposity, have not been replicated (3) and evidence is accumulating that most of the so-called susceptibility genes do not play a direct aetiological role but act as response modifiers to exogenous factors (4). A good example is the role of the C677T polymorphism in the gene encoding the folate-metabolizing enzyme methylenetetrahydrofolate reductase (MTHFR), where those homozygous for the unusual T variant appear to have a lower risk of bowel cancer, but only when intakes of folate are low and/or alcohol intakes are high (5).

The observations by Barker (6) and others that low birth weight (LBW) is associated with increased risk of coronary heart disease, stroke, hypertension and T2D has focused attention on the implications for long-term health of LBW. The risk of T2D (and other diseases) appears to be amplified in those born small who experience accelerated childhood growth and who have greater abdominal adiposity in adulthood (7). These and other observations underpin the concept of "programming", which describes the impact of a stimulus or an insult during a critical or sensitive time-window, resulting in long-term structural and/or functional changes in the organism (8). Programming is an example of developmental plasticity, i.e. that a given genotype can give rise to different phenotypes depending on environmental conditions (9).

Despite the current focus on early life determinants, the impact of adult lifestyle on health (or disease) should not be underplayed. Large-scale, well-designed cohort studies such as EPIC continue to provide robust evidence that food choices and/or nutritional status influence the risk of cancers (10) and affect longevity (11,12). In addition, evidence for lower risk of bowel cancer (and other diseases) in those who undertake greater amounts of physical activity continues to accumulate (13).

Towards effective interventions

Despite the relative wealth of epidemiological evidence that dietary choices (and other lifestyle factors) are associated with disease risk, there is very limited evidence demonstrating the efficacy of lifestyle interventions. Perhaps the strongest evidence comes from randomized controlled trials (RCTs) of the effects of diet and exercise in the prevention of T2D. The Da Qing IGT and Diabetes Study in China showed that dietary and/or exercise interventions in those with impaired glucose tolerance (IGT) reduced the risk of T2D by 31–46% (14). Further large, well-designed RCTs in Finland (15) and the USA (16) produced evidence of even larger benefits of lifestyle modification in similar high-risk IGT subjects.

If the tenets encapsulated in the health pendulum (Fig. 1) are defensible, then there may be utility in designing interventions that target those with particular genotypes, those with LBW and/or those showing greater than expected weight gain in childhood. Most of the studies to date investigating potential diet–gene interactions are observational and where there have been interventions, genotyping was undertaken retrospectively [e.g. Grimble et al. (17)]. While the idea that knowledge of an individual's genetic profile could be used to tailor lifestyle changes that would reduce disease risk has been widely touted, the evidence base for such interventions is very limited (18). In addition to the formidable design (and possible ethical) challenges that such a research strategy entails (19), there is as yet no evidence that knowledge of one's genotype would be a positive motivator for lifestyle change (20). Gibney and Gibney (21) have argued that it will be easier to obtain evidence of efficacy of diet–gene interactions in the clinical management of disease with later applications in public health nutrition.

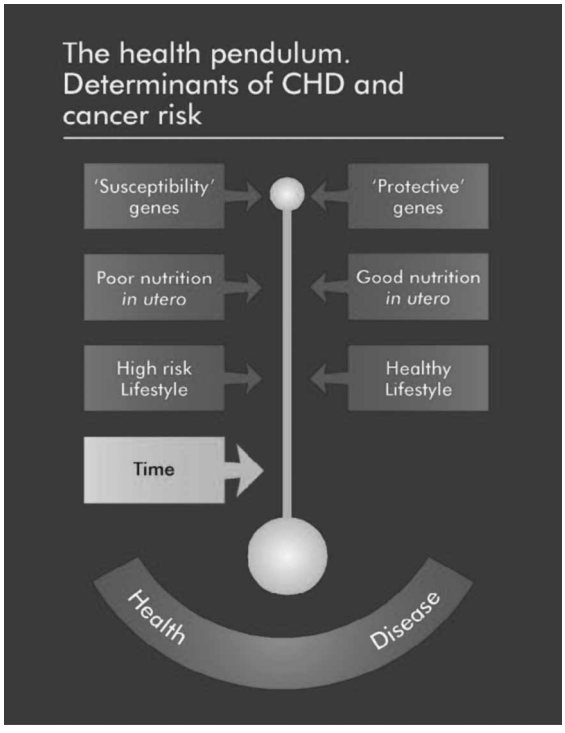

Fig. 1. The health pendulum [adapted from Mathers, 2002 (2)].

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Animal models in human nutrition research

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Introduction

Nutrition can be defined as the "processes by which an organism takes up food and digests, metabolizes and assimilates its nutrients to sustain body function". This definition covers a large range of different physiological processes and these are closely connected with both short- and long-term health. Conversely, an animal model can be defined as "a living organism in which normative biology or behaviour can be studied, or in which a spontaneous or induced pathological process can be investigated, and in which the phenomenon in one or more respects resembles the same phenomenon in humans or other animal species" (1). An ideal animal model for humans will then be one that shows a high similarity with the same phenomenon in humans. The validity of the model may be defined as predictive validity (i.e. extent to which performance in the animal predicts performance in humans), face validity (i.e. extent to which the phenomenon in animals is analogous to humans) or construct validity (i.e. extent to which the animal model has a sound theoretical rationale).

Nutrition is a large field covering an enormous range of complex food components and physiological phenomena, which cannot be studied using a single animal model. To improve knowledge and understanding in nutrition research, animal models need to extend from basic mechanistic models of specific metabolic processes to models aimed at investigating health or disease effects of intact diets. Despite the increasing public concern and ethical constraints regarding use of experimental animals, there is little doubt that animal models in human nutrition research will continue to be an essential element to complement classical food science research and human intervention trials in identifying the mechanistic relationship between diet and health. What then are the basic requirements for a relevant animal model in nutrition research?

Natural dietary habits and the structure of the digestive system

Across all mammalian species, the structure and function of the gastrointestinal tract is the main determinant of the natural dietary habits and the nature of the absorbed nutrients. This makes it a difficult task to identify relevant animal models in nutrition research. Predominantly herbivorous species are equipped with extensive microbial capacity to ferment the slowly digestible plant material, either in the proximal gastrointestinal tract (e.g. a rumen in cattle and sheep) or in the hind gut (e.g. a large sacculated caecum or colon in horses, rats and non-human primates such as baboons). In these species, dietary protein and fat undergo modification by the gastrointestinal microflora and extensive carbohydrate fermentation results in marked absorption of short-chain fatty acids (SCFAs). In contrast, predominantly carnivorous species (e.g. dogs, cats, mink) have a much smaller and shorter gastrointestinal tract and limited bacterial fermentation. Food digestibility is high and nutrient absorption accurately reflects the high protein and fat contents in diet. Humans and other omnivorous species, such as the pig, have gastrointestinal tract characteristics that are intermediate between the above two extremes. In addition to the differences in the nature of absorbed nutrients, even from a identical diet, the species-dependent gastrointestinal tract anatomy induce a large series of functional, microbiological and immunological differences that may influence the tissue and organ responses to absorbed nutrients. Ruminant species (e.g. cattle, sheep) may therefore be poor nutritional models for adult humans, partly because of their large capacity to ferment dietary fibre and absorb SCFAs, while carnivorous species are poor nutritional models because of their limited ability to digest starch and fibre-rich diets. In contrast to humans, both groups of species absorb a limited portion of their diet as glucose, and both are relatively glucose intolerant (type 2 diabetic). In humans, glucose intolerance is a major health problem, and is partly related to deficient dietary fibre intake. This example shows that we must be very careful in selecting animal models to study nutrition-relevant biological phenomena in humans.

Spontaneous or induced nutrition–disease models

Animal models may be spontaneous, induced or negative. In spontaneous animal models the phenomenon in question occurs spontaneously, probably because animals have been

selected genetically over generations on the basis of specific mutations leading to an increased incidence of this phenomenon, independent of nutrition. Well-known examples are classical rodent models for diabetes mellitus, e.g. the selectively bred type 1 diabetic NOD mouse (2) or the type 2 diabetic obese mouse derived from recessive mutation of the *ob* gene marker (3). Within the past 20 years, transgenic technology has enabled the insertion of genes in a range of animal species (4) and the knockout of genes, primarily in mice (5), but recently also in other species such as pigs (6) and rats (7).

In induced animal models some artificial exogenous factor (e.g. malnutrition, pharmacology, surgery) is used to induce the phenomenon in question, e.g. when minipigs are fed *ad libitum* with a diet of high caloric density to induce metabolic syndrome (8). A negative animal model is used to study a biological phenomenon that is normally present in humans, to clarify alternative pathways or the reasons for the absence of this phenomenon. As an example, the pig, in contrast to humans, does not activate factor VII when fed animal lipids (9).

Nutrition, body growth and organ development

Nutrition plays a major role during the entire life cycle, from embryogenesis and foetal development to postnatal growth, adulthood and the degeneration phase. Long-term nutrition studies, and their relation to health and disease patterns during the life cycle, are very difficult to perform and also very expensive. Animal models are therefore an attractive alternative, but to what extent are they relevant in a human developmental context? Their lifespan is much shorter, and the developmental timing of critical nutrition transitions, such as birth and weaning, varies widely among species. As with most basic research, rodent models have been most commonly used to study nutrition effects during the life cycle. However, the short lifespan and the timing of organ and tissue maturation, and the influence of nutrition on development, are fundamentally different in rodents compared with humans. These differences make the study of complex developmental processes difficult. Hence, more analogue animal models should be applied to avoid misconceptions about body developmental processes and the effects of diet and nutritional status (10).

Rodent models

The genetic background of laboratory rodents is usually determined by inbreeding for more than 20 generations, resulting in low biological variation and high reproducibility. However, different strains of inbred mice may have to be applied to study the same phenomenon, as one strain may only resemble the phenomenon in some humans. For example, when fed the isoflavone daidzein mice are known to have a high enteric turnover to equol (11), while this is subject to a higher individual variation in humans (12). Rodents are easy to house in high numbers under standardized conditions. Therefore, the advantage of using rodents is that large group sizes with little interanimal variation can be used and therefore a high power of the study can be achieved and even small biological differences can be elucidated. Another advantage is the availability of a huge number of specific disease models and assays (e.g. DNA array chips) to address specific biochemical parameters in rodents. The disadvantage is their small size, which prevent many experimental manipulations, and that they may also suffer from some absence of nutritional and developmental analogy with humans.

Porcine models

Like humans, the pig is an omnivorous species. The natural diet is a mixture of products of both animal and vegetable origin and a major fuel in energy metabolism based on degradation of starch- and oligosaccharide-containing diets and subsequent hexose absorption. The gastrointestinal tract has many anatomical similarities to that in humans, although the volume capacity of the caecum–colon region is somewhat larger. Correspondingly, the pig is slightly more herbivorous than humans and thus better adapted to a high-fibre diet. Regardless, the pig meets some of the shortcomings of rodent models in nutrition research. Pigs may be even better models than non-human primates, such as baboons, because the latter are relatively herbivorous and thus have diverging characteristics in nutrient absorption and metabolism. The pig has also become an increasingly popular model in developmental nutrition research (10) as well as in studies on adult hypertension, type 2 diabetes and obesity (8, 13). Although genetic manipulation tools and assay availability are less advanced than for rodents, this field is rapidly escalating. In some aspects minipigs may prove to be a better spontaneous nutrition–disease model than conventional pigs because of their slower growth and greater tendency for fat deposition.

Conclusions

Regardless of the choice of animal species in nutrition research, great care is required when results obtained in animals are extended to humans. Many misconceptions have been based on wrong interpretations of results obtained in animals as scientists frequently have disregarded that animal models are selected on the basis of their analogy with one single phenomenon, and as such cannot be applied for interpretations beyond this. Furthermore, a response to an experimental (e.g. dietary) factor is often confined to the animal model only. This problem is also valid for the field of human nutrition and its relation with health and disease in the short and long term. However, animal studies have proven to be useful, and even essential, in obtaining a lasting understanding of the mechanisms involved in the nutrition–disease relation. This valid understanding of nutrition effects in the human body cannot be based alone on more reductionistic experimental approaches such as nutrient effects in organ, tissue or cell culture systems. Thus, animals will provide us with food as well as with the important tools that tell us how food interacts in the human body to promote growth, health and, potentially, increased disease susceptibility.

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Statistical power, personalised nutrition and ethics

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Proposals for the marketing of personalised nutritional advice presuppose population research, biobanks and statistical power. In the context of ethical discussion of genetic databases, much attention has been paid to questions of informed consent, privacy and confidentiality of genetic information. The issues of upstream and downstream public engagement have also been central, as genetic literacy becomes associated with contemporary interpretations of citizenship. Less attention has been given, however, to the *ethical* aspects of the statistical power of these initiatives. Although there has been some debate about the statistical power needed for, e.g., UK Biobank to achieve its objectives, the specifically ethical dimensions of this question have received little attention. It is the aim of this paper to broaden the scope of ethical debate to address this, with special reference to the political and commercial objectives of 'personalisation' of nutritional advice. What are the ethical requirements for an evidence-base for personalisation? In order to address this question it will be necessary to address the different meanings of personalisation, the renegotiation of the relationship between individual and collective in the context of biobanks, and the prospects for realising the promises of the personalisation project.

Weight management through foods providing a metabolic advantage

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Introduction

Increasing recognition of and concern about the recent global rise in overweight/obesity have prompted exploration of dietary and behavioral means to prevent, ameliorate or reverse the problem. There is little doubt that total energy restriction and/or increased energy expenditure would be effective. However, these approaches have not been highly successful. Food restriction results in unpleasant appetitive sensations and decreased quality of life leading to poor dietary compliance. Physical activity can promote negative energy balance, but requires a time commitment incompatible with many people's lifestyles. Thus, much recent work has sought to identify foods or dietary components that can be manipulated to promote energy balance or even negative energy balance without confronting these obstacles. Among the recent targets are soup; nuts; dairy products; tea; foods that have a low glycemic index, high fiber, high protein and/or require mastication; as well as meals that are monotonous or small. This list is comprised of items with properties that fall into three functional categories: (i) enhances satiety; (ii) decreases efficiency of energy absorption; and (iii) augments energy expenditure. Theoretically, items that possess one or more of these properties offer a metabolic advantage that can be exploited as an aid to weight management. A critical review of the literature is provided to aid assessment of the potential for this approach.

Enhanced satiety

Satiation is defined as those sensations that determine meal size as measured by the weight, volume or energy content of ingested food or by the duration of an eating

occasion. In contrast, the sensations that determine the intermeal interval, i.e. eating frequency, constitute satiety. Arguments have been made that the primary problem regarding energy balance lies either in greater meal size (1) or eating frequency (2). Regardless, it is necessary to address both sets of sensations in any weight management plan, otherwise one may compensate for the other. This need not be achieved through any one food constituent or whole food. Total diet is the relevant unit for energy modification. A wide array of foods or food constituents has been singled out based on a purportedly strong influence on appetitive sensations relative to their caloric contribution. They will be considered singly, but it may be that combined they will yield added benefit.

Soups are consistently reported to hold strong satiety properties, but an explanation has been elusive (3). Attributes such as serving temperature, volume, weight or low energy density do not account for this phenomenon, but cognitive effects may be responsible (3–5). It is hypothesized that soup is viewed as a substantive caloric meal component that gives the impression of expanding the size of an eating occasion with resulting higher satiation/satiety ratings. There are preliminary data suggesting inclusion of soup in the diet is effective for promoting weight loss (6).

Evidence from epidemiological studies (7, 8) reveals an inverse relationship between the frequency of nut consumption and body mass index (BMI). This is supported by data from intervention trials (9–11) and is attributable, in large part, to the high satiety value of nuts. Studies across a variety of nuts (e.g. almonds peanuts, pecans) reveal they elicit strong dietary compensation (9–11). Approximately two-thirds of the energy from nuts is offset by unwitting decreases in energy intake at other times of the day. Their inclusion in the diet not only fails to promote weight gain, but may also facilitate weight loss (12). The latter observation is probably attributable, in part, to better dietary compliance stemming from enhanced diet variety and palatability (13).

The glycemic index (GI) is a property of food that reflects the blood glucose response (GR) that it elicits (14). The glycemic load (GL) is the product of a food's GI and absolute carbohydrate content. Several preload studies indicate that consumption of high GL meals increases the postprandial glucose and insulin response, leading to a more rapid and greater rebound of hunger and overeating in a subsequent meal relative to low GL meals (15). In contrast, low GL meals purportedly result in slow, prolonged glucose disposal and greater satiety (16). Other work challenges this view (17) and the premise that either glucose or insulin is causally related to appetite regulation (18). The link between GI score and satiety may reflect other common properties of low GI foods such as their high protein or fiber content.

Fiber is widely recognized as a satiation/satiety factor in foods (19). It may exert this effect by increasing mastication, gastric distension and gastrointestinal transit time (soluble fiber) and by delaying nutrient absorption. An unanswered question is the strength of the effect. Often, to demonstrate its satiety effects, 20–35 g portions of fiber are administered in a preload or meal and appetitive sensations are monitored for several hours. However, this dose far exceeds the daily level in most Western diets, so its practical implications are uncertain. Further, questions about its long-term efficacy are open. One review of the literature reported that 20% of trials using fiber supplementation through foods failed to demonstrate beneficial effects on appetite. High-fiber diets have led to as much as a 10% reduction in energy intake, but whether this was due to enhanced satiety is not known. High-fiber diets also tend to be lower in fat and energy density.

There is a hierarchy of satiety effects for the macronutrients: protein > carbohydrate > fat > ethanol. However, there are caveats to this scheme. For example, when equated for energy density, carbohydrate and fat behave similarly (20). In addition, the food matrix can accentuate or diminish effects. The high satiety value of protein is largely lost when consumed in fluid form (21). Nevertheless, protein does appear to hold the strongest satiety property of the macronutrients and this may explain part of the short-term success of high-protein diets. Monotony is also a likely contributor. Whether there is a linear dose–response relationship between protein and satiety and whether it holds at low concentrations must still be determined. Questions about whether the source of protein is important are also unsettled. There are some reports of higher satiety with dairy protein, possibly due to caseinomacropeptide (CMP), a breakdown product of diary proteins (22,23). However, other studies reported no effect of CMP on appetite (24).

Beverages have been identified as problematic for weight gain because they have low satiety value and prompt little or no dietary compensation (25). This is highlighted by the stronger effects observed when the same foods are masticated (26). It has been hypothesized that the mechanical act of chewing generates a satiety signal, but it is likely that other properties of solid foods (e.g. effects on gastric distension, gastrointestinal transit, rate of absorption) also contribute. The role of mastication warrants further study because chewing efficiency may alter the bioavailability of nutrients that elicit endocrine responses as well as nutrient absorption rates and efficiency, with implications for satiety.

Dietary variety has been a longstanding nutritional goal as it promotes the ingestion of adequate and not excessive concentrations of needed nutrients. Concern has been raised that this advice encourages overconsumption. This is supported by some (27,28), but not all (29) studies. To confuse the issue further, the effects of variety on intake may depend on the food group of interest. Ingestion of greater variety among vegetables is associated with lower energy intake. whereas the reverse was true for the sweets and snacks grouping (28). Taken to the extreme, monotony has been associated with reduced energy intake, but the desirability of dietary variety is so strong that rarely are individuals willing to adhere to diets based on this principle for long periods.

There is a common belief that eating fewer, larger meals promotes positive energy balance. This stems from ob-

servations of an inverse association between self-reported eating frequency and BMI or body fat. However, there are sound alternative explanations (30). First, the association may be due to reduced eating frequency by heavier individuals as a means to reduce body weight. It may also be an artefact of underreporting and omission of eating occasions, which are more common among the overweight/obese. While there may be health benefits associated with increased eating frequency of smaller meals, the data are not compelling that this strategy influences energy balance.

Decreased efficiency of energy absorption or use

Foods with lower energy bioaccessibility may aid in weight management by permitting a greater volume or weight of food intake, thereby ameliorating hunger, while still promoting negative energy balance. Perhaps the longest recognized food component with this property is fiber. Fiber reduces the bioavailability of fat and protein. Losses in the order of 5% of energy occur with higher fiber foods, but this may be partly offset by fermentation of fiber in the colon (19).

Dairy product consumption has recently been associated with lower body weight or accelerated weight loss in several (31,32), but not all (33) studies. Proposed mechanisms have included enhanced satiety, increased lipolysis and fat oxidation as well as calcium soap formation. The latter entails binding of fatty acids with calcium in the gastrointestinal tract resulting in insoluble calcium–fatty acid soaps with reduced absorption. In a randomized cross-over trial of three isocaloric diets varying in calcium and protein, fecal fat excretion increased about 2.5-fold, up to about 18% of ingested fat, on a high-calcium/low-protein diet compared with high-calcium/high-protein or low-calcium/normal-protein periods (34). Making assumptions about constant energy expenditure and dietary intake, this would correspond to a weight loss of 3.5 kg $year^{-1}$. While this work entailed a level of calcium intake (1735 mg day^{-1}) that markedly exceeds that of most consumers (generally <800 mg day^{-1}) and the effects were greater than reported by other groups previously, there may be a contribution of decreased fat absorption to the reported effects of dairy consumption on BMI.

The high satiety value of nuts contributes to their weak or inverse association with BMI, but other factors are also involved. Intervention trials with nuts reveal that daily energy intake is higher when nuts are incorporated in the diet (9,10). Thus, there must be a mechanism for dissipation or loss of that incremental intake. One contributor is low lipid bioaccessibility from this food. Clinical trials document increased fecal fat concentrations among nut consumers. This occurs because of incomplete mastication and resistance of the cell walls to enzymic or bacteriological degradation (35). Thus, the lipid remains encased and unavailable for absorption. Taken together, the evidence suggests that 10–20% of the energy from a variety of nuts is lost in the stool.

Increased energy expenditure

Negative energy balance may be achieved by increased physical activity, but this requires the expenditure of a valuable personal commodity, time. The acceptability of this approach to energy balance has been low among much of the population. However, there are alternative means to increase energy expenditure that do not require a trade-off with time. Protein has a high thermogenic effect (36). That is, the cost of digesting and assimilating protein is higher than for the other macronutrients. Excellent reviews of this effect have been published (36) and suggest that a minimal effect is possible. Doubling protein from 15% to 30% of energy could result in an increased energy expenditure of about 23 kcal day^{-1}, slightly less than 1% of daily energy expenditure. Assuming that no other form of compensation occurs, this marked dietary change could result in about a 1 kg $year^{-1}$ weight loss by this mechanism.

Tea is a rich source of polyphenolic compounds (40% of leaf dry weight). Among the most abundant are catechins. In addition to their antioxidant properties, these compounds reportedly act synergistically with caffeine to enhance sympathetic activity (37). As a result, they enhance thermogenesis. This may serve to moderate appetite and weight gain. Some *in vitro* (37) and human studies (38) suggest that thermogenesis and fat oxidation are enhanced with green tea extract, but there are conflicting data (39). A substantive question is whether the tea polyphenolics are bioavailable in humans.

Conclusions

Maintenance of energy balance is proving to be difficult for the majority of the population. There are foods and food constituents that may aid this effort by offering some metabolic advantage. However, the effects are small and often apparent only under specific conditions of uncertain acceptability to consumers. None appears to offer the simple, rapid, painless fix sought by many consumers. Whether synergies exist between these factors that may offer greater benefit is worthy of further exploration.

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Parallel sessions 1–3, 15 March 2006
Oral presentations

Parallel session 1: Biomarkers and prevention of lifestyle-related diseases

Bertram HC. ^{1}H-NMR-based metabonomics as a tool to elucidate the health benefits of wholegrain cereals

Kristensen M. Short-term effects on insulin-like growth factor-1 of replacing milk with cola beverages: a 10 day interventional study in young men

Olsen A. Plasma enterolactone and risk of postmenopausal breast cancer according to oestrogen receptor status

Krogholm KS. Flavonoids in human morning urine and 24 hour urine as biomarkers for intake of fruit and vegetables

Parallel session 2: Animal models in human nutrition research

Licht TR. Dietary carbohydrate source affects intestinal microbiota and short-chain fatty acid composition in rats

Lawson MA. Development of a cellular assay from rats for fundamental studies on early events in taste perception

Siggers RH. Diet-induced intestinal mucosal atrophy and dysfunction in preterm pigs is bacteria dependent

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Parallel session 3: Civilization, ethics and marketing

Castle D. Invited speaker. Nutrigenomics and personalization: Science quality, ethics, and regulation (*Abstract will be distributed at the congress*)

Ebbesen M. Nutrigenomics: lessons to be learned from the debate on genetically modified foods?

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Parallel sessions 1–3, 15 March 2006
Abstracts

^{1}H-NMR-based metabonomics as a tool to elucidate the health benefits of wholegrain cereals

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Objective: The aims were to examine ^{1}H-nuclear magnetic resonance (NMR)-based metabonomics as a tool to elucidate the biochemical effects in blood and urine after the consumption of wholegrain products using the pig as a model.

Methodology: Two diets with similar levels of dietary fibre and macronutrients, but with contrasting levels of plant phytochemicals, were prepared from rye (high in phytochemicals, RD) and wheat (low in phytochemicals, WD) and fed to four portal vein and six urinary-bladder catheterized pigs in a cross-over design. Plasma and urine samples were collected after 5–7 days on each diet. ^{1}H-NMR spectra were acquired on a Bruker Avance 400 MHz spectrometer. Partial least squares regression discriminant analysis (PLS-DA) on spectra obtained for plasma and urine samples were used to identify differences between diets, which was followed up by liquid chromatography–mass spectrometry (LC-MS) analysis to verify the differences.

Results and discussion: PLS-DA on spectra obtained for plasma samples revealed that the spectral region at 3.25 ppm dominated the differentiation between the two diets, as the RD diet was associated with higher spectral intensity in this region. Spiking experiments and LC-MS analyses of the plasma verified that this spectral difference could be ascribed to a significantly higher content of betaine in RD plasma samples compared with WD samples. In an identical study with the same diets, urine samples were collected, and ^{1}H-NMR spectra were acquired on these. PLS-DA on spectra obtained for urine samples revealed changes in the intensities of spectral regions that could be ascribed to differences in the content of betaine and creatine/creatinine between the two diets, and LC-MS analyses verified a significantly lower content of creatinine in RD urine samples compared with WD samples.

Conclusions: The present study demonstrated that ^{1}H-NMR-based metabonomics is an excellent tool for exploring the biochemical effects of a diet with a high content of wholegrain cereals, which can contribute to elucidating the health benefits of a wholegrain-based diet.

Short-term effects on insulin-like growth factor-1 of replacing milk with cola beverages: a 10-day interventional study in young men

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Objective: In the Western world, increased consumption of carbonated soft drinks combined with a decreasing

intake of milk is observed. This may affect circulating insulin-like growth factor-1 (IGF-1) and fasting insulin, as seen in prepubertal children. High levels of circulating IGF-1 are associated with increased risk of hormonal cancers, whereas low levels are associated with cardiovascular disease. High levels of fasting insulin and insulin resistance are associated with increased risk of developing type 2 diabetes mellitus (T2DM). This intervention study was designed to reflect the trend for replacing milk with carbonated beverages in a group of young men and to study the effects of this replacement on IGF-1, IGFBP-3 (binding protein) and the molar ratio of IGF-1:IGFBP-3 and on glucose–insulin metabolism.

Methodology: The study was a randomized, controlled cross-over intervention study, in which 11 men aged 22–29 years were fed a low-calcium diet in two 10 day periods with 10 day washout in between. In one period they drank 2.5 litres of Coca Cola® per day and the other period 2.5 litres of semi-skimmed milk with the same energy content. Serum concentrations of IGF-1, IGFBP-3 (RIA), insulin (FIA) and glucose (Cobas) were determined at baseline and the end-point of each intervention period. Insulin resistance and β-cell function were calculated with the homoeostasis model assessment (HOMA).

Results: An decrease in serum IGF-1 ($p < 0.05$) was observed in the cola period compared with the milk period. No changes were observed in serum concentrations of IGF-1:IGFBP-3, insulin, insulin resistance or β-cell function. IGFBP-3 ($p = 0.19$) and glucose ($p = 0.20$) increased similarly in both periods.

Conclusions: This study demonstrates that over a 10 day period high intake of cola decreases total IGF-1 compared with a high intake of milk, with no effect on glucose–insulin metabolism. It is unknown whether this effect is a transient phenomenon or whether it has long-term consequences.

Plasma enterolactone and risk of postmenopausal breast cancer according to oestrogen receptor status

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Objective: Phyto-oestrogens, including enterolactone, have been hypothesized to prevent breast cancer. Research within the field has, however, been sparse. One of the biological effects of enterolactone is probably an antioestrogenic effect. It is therefore likely that the breast cancer preventive effect interacts with the oestrogen receptor status of the tumour. The aim was to investigate whether plasma levels of enterolactone provide a preventive effect on breast cancer risk and to explore whether the relation differs with the oestrogen receptor status of the tumour.

Methodology: From 1993 to 1997, 29 785 Danish women aged 50–64 years were included in the cohort "Diet, Cancer and Health". Information about diet and lifestyle factors was obtained by questionnaire and blood was drawn. During follow-up, 381 postmenopausal women were diagnosed with breast cancer. The breast cancer cases were matched to 381 controls. Levels of enterolactone in plasma were analysed by time-resolved fluoroimmunoassay. Associations between plasma levels of enterolactone and breast cancer risk were analysed using Cox's regression model.

Results and discussion: The incidence rate ratio (IRR) for all breast cancer was 0.94 [95% confidence interval (95% CI) 0.87–1.01] per 20 nmol l^{-1} higher plasma level of enterolactone. For oestrogen receptor-positive cancers no association was seen (IRR = 0.97, 95% CI 0.89–1.06), whereas a significant protective effect was seen on oestrogen receptor-negative cancers (IRR = 0.73, 95% CI 0.56–0.95) per 20 nmol l^{-1} higher plasma enterolactone. Adjustment for established risk factors for breast cancer did not alter the results.

Conclusions: In accordance with earlier research, there was a tendency towards a decreasing risk of breast cancer with increasing plasma levels of enterolactone. The protective effect was almost entirely constrained to oestrogen receptor-negative breast cancer.

The study has been published in Cancer Epidemiology, Biomarkers and Prevention 2004;13:2084–9.

Flavonoids in human morning urine and 24 hour urine as biomarkers for intake of fruit and vegetables

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Objective: To gain better insight in the potential health effects of fruit and vegetables, reliable biomarkers of intake are needed. The main aim of this study was to investigate the sensitivity of flavonoids as biomarkers of fruit and vegetable intake in both 24 h and morning urine in relation to moderate changes in dietary intake of fruit and vegetables.

Methodology: Levels of seven dietary flavonoids (naringenin, hesperetin, phloretin, quercetin, tamarixetin, kaempferol and isorhamnetin) were determined by liquid chromatography–mass spectrometry (LC-MS) in urine samples from 12 male subjects. The study was designed as a randomized, controlled cross-over study providing single doses of either 300 or 600 g of fruit and vegetables.

Results and discussion: The study showed that the total excretion of flavonoids (the sum of all seven flavonoids) in 24 h urine samples increased in a significant, sensitive and linear manner with increasing intake levels of fruit and vegetables ($R = 0.86$, $p < 000001$). The total excretion of flavonoids in morning urine also increased significantly, but was less sensitive than the 24 h urine ($R = 0.59$, $p < 0.0001$). Furthermore, it was shown that the fraction of the total flavonoid dose excreted in 24 h urine as a percentage of intake was unaffected by the changes in the fruit and vegetable intake.

Conclusions: This study validates that urinary flavonoids can be used to study the possible health protective effects of flavonoid intake, that the sum of seven different flavonoids in 24 h urine samples may be used as a new and sensitive biomarker for fruit and vegetable intake, and that the sum of seven different flavonoids in a single morning urine sample probably does not have the potential as a general biomarker for fruit and vegetable intake.

Dietary carbohydrate sources affects intestinal microbiota and short-chain fatty acid composition in rats

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Objective: It has previously been shown that the nature and digestibility of dietary carbohydrates affect azoxymethane-induced colon carcinogenesis in rats. The aim of this study was to elucidate whether effects on the intestinal microbiota may represent a mechanism through which the carbohydrate composition influences the process of carcinogenesis.

Methodology: Five groups of eight rats were fed a Western type diet containing cornstarch (reference group), sucrose, potato starch, Raftiline (a long-chain fructan) or Raftilose (a short-chain fructan). Caecal weight and caecal pH was measured, and the caecal contents of acetic, propionic and buturic acids were estimated by capillary zone electrophoresis. Profiles of the bacterial microbiota were obtained by denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR)-amplified bacterial DNA as well as of reverse transcriptase–PCR-amplified bacterial rRNA extracted from faeces. The profiles were compared by principal component analysis (PCA). Bacterial species representing bands of specific interest were identified.

Results and discussion: The animals fed with the diets containing potato starch, Raftiline and Raftilose had a significantly higher caecal weight compared with the reference group, indicating increased fermentation, and selective cultivation from faeces revealed a higher amount of *Lactobacillus* spp. in these animals. In addition, the fructan groups had a lower amount of coliform bacteria in the faeces. In the Raftiline and Raftilose groups, higher levels of butyrate and propionate, respectively, were measured. DGGE profiles showed a different microbiota in each of the five animal groups. Comparison of DNA-based and RNA-based profiles revealed that two species within the phylum Bacteroidetes had a particularly high ribosome content in the animals fed with Raftiline, indicating that growth of these species were specifically stimulated by this particular fructan.

Conclusions: The gut microbiota of animals was clearly influenced by the type of carbohydrate in their diet, and the previously observed effects on carcinogenesis may therefore partly be attributed to effects on the gut microbes.

Development of a cellular assay from rats for fundamental studies on early events in taste perception

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Objective: The aim of this work was to develop a method using microscopy and a rat model system for the investigation of taste cell signalling in intact taste buds.

Methodology: Sprague–Dawley rats were killed and the upper epithelial layer containing the taste buds was removed using a papain treatment. The tissue sample was then divided into a number of segments and exposed to a specific concentration of sucrose. The tissue was fixed and immunostained for known signal transduction proteins with differing phosphorylation states. Using confocal laser scanning microscopy, differences in protein expression and phosphorylation were then measured using quantitative immunofluorescence to evaluate cell signalling.

Results and discussion: Tongue epithelial sections from Sprague–Dawley rats were removed from excised tongues, each containing a population of taste buds that could be recognized using fluorescent imaging of cell membranes. The taste buds were observed to be intact after the enzyme treatment. Signal transduction through sucrose receptors in taste buds is thought to be a G-protein-coupled event (Montmayeur and Matsumi, Curr Opin Neurobiol 2002;12:366). Signalling pathways after ligand occupancy of these receptions is thought to occur through a mechanism that involved ERK-2 phosphorylation (Ozeck et al., Eur J Pharm 2004;489:139). Therefore, after exposure to buffer with varying concentrations of sucrose, the tongue epithelial sample was immunostained for ERK2 and phospho-ERK2, in conjunction with rhodamine to visualize cell membranes and DAPI to identify cell nuclei. Confocal laser scanning micrographs show an increase in ERK2 phosphorylation after the taste buds have been exposed to $\geq$150 mM sucrose, while the total amount of ERK2 is unaffected. Signal transduction pathways in taste cells are still poorly understood. The difficulty in isolating pure cell populations makes biochemical investigation impractical. The strength of microscopic techniques is the ability to visualize protein changes in specific cells of interest without physical isolation. The ability to follow specific signal transduction events in these cells is an important step in unravelling the complex mechanisms of taste.

Conclusion: A rat model system is well suited to investigating taste cell signal transduction pathways.

Diet-induced intestinal mucosal atrophy and dysfunction in preterm pigs is bacteria dependent

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Objective: Preterm neonates fed milk formula commonly experience small intestinal mucosal atrophy and dysfunction, often increasing their susceptibility to intestinal diseases such as necrotizing enterocolitis (NEC). It is unknown whether this negative intestinal response is exclusively dependent on diet or mediated by factors such as bacterial colonization. A preterm gnotobiotic pig model was used to determine whether small intestinal atrophy and dysfunction in the preterm intestine are bacterial dependent.

Methodology: Thirty-eight preterm pigs (93% gestation) were delivered via caesarean section and reared in either germ-free (GF) or conventional (CV) isolators for 40–48 h. Pigs were fed either infant milk formula (FORM) or sow's colostrum (COL). Germ-free status was confirmed by negative aerobic and anaerobic cultivation.

Results and discussion: Pathological NEC lesions were present in some CV-FORM but absent in all GF-FORM and CV-COL pigs. Relative to CV-FORM, mucosal weights, villus heights and aminopeptidase A and N activities were increased in the GF-FORM pigs ($p < 0.01$). Values were similar to those in CV-COL pigs. Disaccharidase activities were less bacteria dependent, as both groups of FORM pigs (GF and CV) showed markedly lower activities for maltase and lactase, relative to COL pigs ($p < 0.01$).

Conclusions: The negative response to formula feeding in the small intestine of preterm neonates is not exclusively diet induced, but is partly mediated by bacterial colonization of the small intestine immediately after birth.

Rye bread reduces plasma cholesterol levels in hypercholesterolaemic pigs

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Objective: The aim was to investigate how a diet rich in rye fibre compared with a high-fibre wheat diet influences cholesterol metabolism in pigs.

Methodology: Seventeen hypercholesterolaemic pigs were fed wheat or rye bread for 9–10 weeks. The breads had equally high protein, fat, cholesterol and dietary fibre content (20%), but varied in the composition of DF and content of plant lignans (3 vs 105 nmol g^{-1} dry matter). Faeces and urine were collected quantitatively for 7 days after 7–8 weeks, and gastrointestinal contents were collected at the end of experiment to determine ileal viscosity, intestinal digestibility and fermentation profile. Mammalian lignans were determined in plasma, bile and urine. Liver samples were analysed by reverse transcriptase–polymerase chain reaction for the expression of LDLr, HMGR and SREBP2, three genes that are considered important for cholesterol metabolism.

Results and discussion: After 9–10 weeks the pigs fed rye bread had cholesterol levels 60% of those fed wheat breads. In addition, low-density lipoprotein (LDL) levels were lower, changing the LDL:HDL ratio in favour of high-density lipoprotein (HDL). The rye breads increased the viscosity of ileal contents, which might have led to increased excretion of bile acids. This corresponded with a reduction in the ileal and faecal digestibility of crude fat. Furthermore, levels of enterolactone in plasma, bile and urine were 10–25 times as high comparing rye with wheat bread-fed pigs. Owing to a high fermentability of the added cellulose, differences in fermentation pattern cannot explain the pronounced effect on cholesterol metabolism alone. In pigs fed the rye-based diets the relative expression of LDLr, HMGR and SREBP2 in liver tissue was 1.25, 1.22 and 1.21, respectively, compared with wheat (1.00), but changes were not significant. Thus, the cholesterol-lowering properties of rye are likely to be caused by several independent or synergistic mechanisms; arabinoxylans in rye enhance luminal viscosity and thereby reduce the reabsorption of bile acids, and metabolites (e.g. lignans, alkylresorcinols) released when consuming rye may influence cholesterol metabolism at the liver level.

Conclusion: Rye fibre has a beneficial effect on plasma cholesterol in hypercholesterolaemic pigs that is caused by factors other than fibre level alone.

Nutrigenomics: lessons to be learned from the debate on genetically modified foods?

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The emerging field of nutrigenomics is currently experiencing a boom in the number of published research results and funding. Nutrigenomics is an interdisciplinary science that combines elements of genomics, molecular biology, nanoscience, clinical research and nutritional science to explore the interaction between nutrients in foods and genes which, for instance, may contribute to chronic diseases. The fundamental objective of nutrigenomics is to understand individual nutrient genotypes to design dietary interventions that restore health or prevent disease.

However, researchers and society in general need to be aware of the risk that nutrigenomics may suffer the same destiny as genetically modified (GM) foods, which have been boycotted by consumers. This study explores whether the field of nutrigenomics can learn any lessons from the debate on GM foods.

Some researchers claim that public acceptance of new technology depends on confidence, which is created on the basis of information, education, openness and debate. However, empirical studies point out that information and education are not the only factors influencing public attitudes towards new technology. In the public mind, risks also involve moral considerations, democratic considerations and uncertainties. From these studies we can learn that public information on nutrigenomics should encompass more aspects than specific technical–scientific facts. It should deal with political, sociological and ethical aspects of nutrigenomics to meet the requirements of the public. Several of these aspects belong to the research areas of the humanities and the social sciences.

For instance, the humanities and the social sciences reflect on the objectives we wish to realize by introducing new technology and what kind of values are at stake. The aim these reflections is not to build trust and acceptance in the public, but to make a critical assessment of new technology so that the public can make an informed judgement.

The above considerations show the importance of integrating the humanities and the social sciences in the interdisciplinary approach to nutrigenomics. This may create interdisciplinary research environments, where for instance ethicists and natural science researchers are in daily dialogue, facilitating ethical reflection as an integral part of the research process of nutrigenomics.

Interdisciplinary research between food science, genomics, nutrition and health

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Objective: The aim is to bridge the gap between food science, genomics, nutrition and health.

Methodology, results and discussion: An arena with stakeholders representing the government, consumer, industry, retail and research institutes (food science and medicine/nutrition) was established by personal invitations to a careful selected group of stakeholders aided by a detailed communication map. They were invited to attend three interactive workshops to create a dialogue on the topic Food for better health. The last workshop focused on what enterprises were doing to fulfil World Health Organization recommendations. It revealed a huge knowledge gap in the enterprises within food science, technology, nutrition and health. Food for better health covers a wide research area. The relationship between food science, nutrition and health has been better understood during the past decade, and now the relationships with genomics are in the pipeline. Many new nutrients have been discovered, and their stability through the value chain has been studied. Therefore, an innovation network was started with 16 enterprises in close co-operation with the arena. It was a great success and a new network with 10 enterprises has started. Co-operative ventures with medical research institutes have also been established. In addition, knowledge within food science is being combined along the value chain from farm to consumer health. In close collaboration with leading food industry enterprises research projects are being run addressing topics such as knowledge in raw materials, process optimization, statistics and measuring techniques. Regulation and labelling of food with health claims are an important issue in this matter, and the consumer has a right to accurate information. Transfer of knowledge to the enterprises, in addition to the establishment of innovation networks, is central in these projects.

Conclusions: Interdisciplinary research in close collaboration with food industrial partners is needed to bridge the gap between food science, genomics, nutrition and health, and to implement knowledge into leading food enterprises.

PROP status: a sensible tool for selecting the most sensitive subjects for sensory analysis?

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Objective: This study investigated whether subjects selected for sensory taste panels are more sensitive to 6-propylthiouracil (PROP) than subjects drawn randomly from the Danish population.

Methodology: A ring test with eight sensory laboratories in Denmark ($n = 124$ subjects) was carried out using a ISO threshold test for taste detection and taste recognition. The tastants included PROP, quinine and caffeine (bitter), and sucrose (sweet) at different concentrations. The reference group consisted of randomly chosen subjects ($n = 38$) and a group chosen among fish-eating people ($n = 40$).

Results and discussion: PROP sensitivity is genetically determined and has as a phenotype the number of fungiform papillae on the tongue. A high PROP sensitivity results in an increased sensitivity to certain bitter compounds, sweeteners, oral touch (fat perception) and trigeminal irritation (e.g. capsaicin). Populations can be divided into PROP "non-tasters", "medium tasters" and "supertasters". In the Caucasian population, about 25% are identified as supertasters and 20% as non-tasters. The number of supertasters in the Asian population is almost twice of that in the Caucasian population. Relationships between PROP status with food choice, preference and lifestyle diseases have been reported. Subjects with a high sensory sensitivity are needed for sensory analysis. Therefore, PROP sensitivity may be a relevant selection criterion.

The results of this study showed that 42% of the subjects in the sensory panels were supertasters, compared with the randomly drawn group of subjects with only 26% supertasters. Three panels included predominantly PROP supertasters. Sensitivity to PROP was also positively correlated with sensitivity to sucrose. However, a negative correlation with caffeine and quinine sensitivity was observed. In the group of fish-eating people a large percentage of PROP sensitive tasters was observed.

Conclusions: PROP status appears to be an intrinsic factor in the selection of sensory subjects. However, not all subjects selected for sensory panels are supertasters. More work, including studies on ethnic groups, is needed for appropriate recommendations to sensory panel leaders.

Parallel sessions 4–6, 16 March 2006
Oral presentations

Parallel session 4: Gut, health and immunity

Sangild PT. A high-fat, low-fibre diet affects gastrointestinal growth, function and microbiology in aged pigs

Siggers RH. Probiotics improve gastrointestinal structure and function in preterm pigs

Fink LN. Distinct gut-derived bacteria differentially affect three types of antigen-presenting cells and impact on NK-cell and T-cell responses

Rautonen N. Invited speaker. Pre- and probiotics and intestinal immune responses (*Abstract will be distributed at the congress*)

Parallel session 5: Food for obesity prevention

Sloth B. The effect of PYY_{1-36} and PYY_{3-36} on appetite, energy intake and energy expenditure in obese and lean subjects

Schack-Nielson L. High maternal gestational weight gain is associated with an increased body mass index in childhood and adulthood independent of maternal body mass index

Gregersen NT. A meta-analysis of meal-test studies to elucidate the roles of postprandial insulin and blood glucose responses in appetite regulation in normal-weight and overweight individuals.

Holst, JJ. Invited speaker. Meal-induced release of intestinal appetite regulating hormones (*Abstract will be distributed at the congress*).

Parallel session 6: Future food for health

Hagemann K. Consumer versus expert hazard identification: a mental models study of a functional food ingredient

Korzen-Bohr S. Postmenopausal women and heart disease: acceptability of functional foods as a preventive measure

Jespersen BM. Build Your Food

Poulsen M. Quantitative risk assessment strategies for novel foods (NOFORISK): an EU research project

Parallel sessions 4–6, 16 March 2006
Abstracts

A high-fat, low-fibre diet affects gastrointestinal growth, function and microbiology in aged pigs

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Objective: Dietary factors play a role in the development of epithelial diseases such as gastric ulcer and colorectal adenomas, inflammatory bowel disease and colitis. High-fat diets increase the risk of epithelial disturbance, while cereal and fruit fibres protect against disease. The mechanisms underlying these effects of diet remain poorly understood. Therefore, the short-term gut response to differences in fat and fibre intake were investigated in old pigs. In contrast to laboratory rodents, the digestive system and natural dietary habit in mature pigs are very similar to those in humans.

Methodology: Sows (Landrace × Yorkshire, 3.6 ± 0.2 years, 275 ± 7 kg body weight) were fed the same amount of net energy and digestible nutrients originating from either a high-fat, low-fibre diet (FAT, $n = 6$, 210 g fat and 47 g fibre per kg dry matter) or a more natural high-fibre, low-fat diet (FIBRE, $n = 6$, 32 g fat and 410 g fibre per kg dry matter). After a 90 day feeding period, the gastrointestinal tract was removed and samples were taken along the entire length of the gut for later histological, biochemical, microbiological and gene expression analyses.

Results and discussion: In the FAT-fed sows, the relative stomach and colon weights were significantly reduced (20–30%, $p < 0.05$) compared with FIBRE-fed sows. The pars oesophageal region in FAT-fed sows showed more progressive erosive damage, and the caecum had a lower acidity (pH 6.6 vs 6.1, $p < 0.05$) and an epithelium that appeared inflamed, compared with the FIBRE-fed sows. The microbiology of gut contents showed significantly higher numbers of enterobacteria and yeasts at all sampling sites in the FAT group (up to 200-fold increases, $p < 0.05$), while the caecum–colon lactobacillus counts were significantly lowered, compared with the FIBRE group. Intestinal function was assessed by a series of brush-border enzyme activities, and differences in tissue gene expression were tested by a pig DNA macroarray that included 86 intestinally expressed genes, of which 23 were markers for either the proliferative or the mature epithelial cells of the human colon. Collectively, the results demonstrate that there are distinct differences in some structural and functional characteristics of the gut between sows fed a high-fat, low-fibre diet versus a low-fat, high-fibre diet. Some of these differences may be directly related to the marked changes in the gut microflora.

Conclusions: The gut responses in this model of aged sows could be used in the search for markers of the early onset of some diet-induced gastric or colonic epithelial pathological conditions in humans.

Probiotics improve gastrointestinal structure and function in preterm pigs

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Objective: Gastrointestinal diseases, such as necrotizing enterocolitis (NEC) in the preterm infant, are often precipitated by aberrant bacterial colonization. Early manipulation of the gastrointestinal microbiota in preterm neonates may decrease their susceptibility to gastrointestinal dysfunction and disease. A pig model was used to determine whether probiotic administration during the immediate postnatal period could improve gastrointestinal structure and function in preterm neonates.

Methodology: Twenty-eight preterm pigs (93% gestation) were maintained on total parental nutrition (TPN) for 36 h then enterally fed either sow's colostrum or human milk formula. Pigs were allocated into three groups: colostrum (CLS), formula (FORM), or probiotic plus formula (PROB). PROB piglets received orally administered probiotics throughout the TPN and enteral phases.

Results and discussion: The frequency of NEC-like pathological lesions in the small intestine and colon was lowest in

the CLS group, intermediate in PROB and most frequent in the FORM group. Distal small intestine villus height measurements were increased by 42% ($p < 0.01$) for CLS compared with PROB pigs, while PROB pigs showed a 22% increase ($p < 0.05$) compared with FORM pigs. PROB piglets tended to have the deepest crypt depths compared with FORM ($p = 0.28$) and CLS pigs ($p = 0.05$). Relative dry weights of the small intestine mucosa layer in the PROB and CLS pigs were increased (17–20%, $p < 0.05$) compared with FORM pigs. Similarly, small intestine aminopeptidase A and N activities were increased ($p < 0.05$) for PROB (30%) and CLS pigs (50–60%) compared with FORM pigs.

Conclusion: Probiotic administration during early postnatal life improved the structure and function in the compromised gastrointestinal tract of preterm pigs.

Distinct gut-derived bacteria differentially affect three types of antigen-presenting cells and impact on NK-cell and T-cell responses

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Objective: Gut bacteria are assumed to be essential for the development and maintenance of a balanced immune system. Specifically, stimulation of antigen-presenting cells (APCs) by gut bacteria is important for polarization of the immune response. This experiment was designed to reveal similarities and differences between the reaction patterns of three types of human APC when stimulated with intestinal bacteria. Furthermore, the effect of these APCs on NK cells and T-cells was examined.

Methodology: The APCs used in this study were blood monocytes, blood dendritic cells and dendritic cells differentiated from monocytes. Monocyte-derived dendritic cells constitute a commonly used model of dendritic cell function. The APCs were cultured for 18 h with four different gut bacteria: *Lactobacillus acidophilus* X37, *Lactobacillus reuteri* DSM 12246, *E. coli* Nissle 1917 or *Bifidobacterium longum* Q46.

Results and discussion: To examine the polarizing effect of gut bacteria on APCs, surface markers and cytokines were measured. The co-stimulatory molecules CD40 and CD86 were induced to a different extent together with CD83. Interleukin-12 (a Th1 cytokine) was only induced by *Lactobacillus acidophilus*. Interleukin-10, which promotes the development of regulatory T-cells, was mainly induced by the other bacteria. Interleukin-6 and tumour necrosis factor are proinflammatory cytokines, often induced by pathogens, but also by some gut bacteria. The effect of the four gut bacteria on monocyte-derived dendritic cells has previously been examined, but this study revealed that their effect on other kinds of APCs is markedly different. When APCs matured by different bacteria were added to either NK cells or T-cells, different APCs combined with distinct strains of bacteria caused the production of varying amounts of cytokines.

Conclusions: Distinct gut bacteria possess individual properties leading to different effects on APCs, NK cells and T-cells. Because NK cells play a major role in T-cell polarization, and because the APCs affect T-cells directly, gut bacteria may be very important in maintaining a balanced immune response through these mechanisms. The bacteria examined can potentially be used in tailored probiotic foods exploring their immunomodulatory properties.

The effect of PYY_{1-36} and PYY_{3-36} on appetite, energy intake and energy expenditure in obese and lean subjects

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Objective: Infusions of PYY_{3-36} in humans have been shown to produce dramatic reductions in *ad libitum* food intake, whereas results in animals are conflicting. The aim of the present study was to compare effects of infusions with saline, PYY_{1-36} or PYY_{3-36} on appetite, energy intake, energy expenditure and blood concentrations of PYY, insulin, glucose, free fatty acids (FFA) and triglycerides (TG).

Methodology: Twelve lean [body mass index (BMI) 20–25 kg m^{-2}] and 12 obese (BMI 28–36 kg m^{-2}) subjects were given 90 min 0.8 pmol kg^{-1} min^{-1} infusions of PYY_{1-36}, PYY_{3-36} and saline in a blinded randomized cross-over study. Subjects were fasting before and during the infusion and an *ad libitum* lunch was served 2 h after termination of the infusion. Blood was sampled and appetite sensations were recorded on visual analogue scales every 30 min during the test day. During the first 4 h of the test day energy expenditure and substrate oxidation was measured by indirect calorimetry using a ventilated hood system.

Results: Five out of nine subjects receiving PYY_{3-36} experienced nausea and therefore these infusions were discontinued. The four subjects who completed the PYY_{3-36} test days had a significantly lower *ad libitum* energy intake compared with placebo and PYY_{1-36}, but these subjects also had a lower rating of well-being, although they did not report nausea. In the fasting state infusion of PYY_{3-36}, compared with placebo and PYY_{1-36}, produced a significantly higher FFA concentration and postprandial FFA, glucose and insulin concentrations were also elevated. As for the PYY_{1-36} infusions, no robust effects were seen in energy intake, appetite, energy expenditure or blood concentrations of FFA, insulin, glucose and TG.

Conclusions: The anorectic effects of PYY_{3-36} are likely to be due to discomfort. PYY_{3-36} caused increased lipolysis and increased postprandial insulin and glucose responses. PYY_{1-36} had no effects on energy intake, appetite, energy expenditure or any of the measured blood parameters, which might partly be due to doses being to low.

This study was supported by EC-FP6 (contract no. LHM-CT-2003-503041) and an institutional grant from AdiTech Pharma AB.

High maternal gestational weight gain is associated with an increased body mass index in childhood and adulthood independent of maternal body mass index

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Objective: The aim was to explore the effect of maternal prepregnancy body mass index (BMI) and gestational weight gain (GWG) on offspring BMI in childhood and adulthood.

Methodology: The Copenhagen Perinatal Cohort consists of 9125 individuals born at the Copenhagen University Hospital in 1959–1961. Information on birth weight, gestational age (GA), socioeconomic status (SES) at birth, smoking during pregnancy, maternal age, weight, height and GWG (in five categories assigned the interval middle value) were recorded prospectively at birth and at a 1 year examination. Weight and height were available from follow-up examinations at 1, 3 and 6 years, from school health records at 8, 11 and 14 years, and from a mailed questionnaire at the age of 42–44 years. Regression analysis with BMI in childhood or adulthood as the dependent variable was performed. For 2499 participants information was available to be included in at least one analysis.

Results and discussion: The prevalence of overweight (BMI $\geq$25 kg m^{-2}) and obesity (BMI $\geq$30 kg m^{-2}) was 9% and 1% among the mothers and 43% and 11% among the adult offspring. GWG was 5.5, 7.0, 9.5, 11.5, 14.0 and 16.0 kg for 7, 16, 18, 18, 21 and 19% of the mothers, respectively. Maternal BMI was higher in the lowest GWG group compared with the others (mean $\pm$ SD 22.7 $\pm$ 3.8 vs 21.4 $\pm$ 2.6, $p < 0.001$). In regression analyses adjusted for maternal age, SES at birth, smoking during pregnancy and gender, both maternal BMI and GWG were positively associated with BMI at all ages. In the model with adult BMI as the dependent variable the regression coefficients were $\beta = 0.50$ [95% CI (95% confidence interval) 95% CI 0.41–0.57, $p < 0.001$] and $\beta = 0.10$ (95% CI 0.04–0.15, $p = 0.01$) for maternal BMI (kg m^{-2}) and GWG (kg), respectively.

Conclusions: In a cohort of women with a low prevalence of overweight a positive effect of GWG on offspring BMI was seen that persisted into adulthood. This finding supports the current evidence suggesting that excessive GWG should be avoided.

A meta-analysis of meal-test studies to elucidate the roles of postprandial insulin and blood glucose responses in appetite regulation in normal-weight and overweight individuals

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*AF worked at address 1 while the meta-analysis was conducted.

Objective: It is not clear whether postprandial blood glucose or insulin exerts a regulatory function in relation to short-term appetite sensations and subsequent energy intake, and whether any difference exists between normal-weight and overweight subjects. Therefore, this study investigated these relations using meta-analysis.

Methodology: Data from seven meal-test studies were used, including 136 healthy subjects (ALL) [92 normal-weight (NW), 44 overweight or obese (OW)]. All meals were served as breakfasts after an overnight fast, and appetite sensations and blood samples were obtained frequently in the postprandial period. Finally, an *ad libitum* lunch was served. Data were analysed both by fixed effects study-level meta-regression analysis (SL) and by individual subject data regression analysis (ISD), using STATA.

Results and discussion: In SL analysis, the postprandial insulin response was associated with decreased hunger in ALL, NW and OW ($p < 0.019$) and with increased satiety in NW ($p = 0.004$) and lower subsequent energy intake in OW ($p = 0.022$). Data from multivariate ISD analysis showed the same associations, but only in NW for hunger, satiety and energy intake ($p < 0.028$) and in ALL for energy intake ($p = 0.016$). The only association involving blood glucose was the multivariate ISD analysis, showing an inverse association between blood glucose and subsequent energy intake in ALL ($p = 0.032$).

Conclusions: The results suggest that insulin, but not glucose, is associated with short-term appetite regulation in healthy subjects, but the relation is disrupted in overweight and obese people. The postprandial insulin response is likely to be an important satiety signal, and CNS insulin resistance in overweight may explain the blunted effect on appetite.

Consumer versus expert hazard identification: a mental models study of a functional food ingredient

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Objective: The consumer part of the EU project NOFORISK compares laypeople's and experts' understanding of benefits and risks associated with the functional food ingredient Phytosterol. The Council of the European Union has recently authorized the marketing of Phytosterol-enriched rye bread as a novel food under Regulation (EC) No. 258/97.

Methodology: The methodology used, mental models, is relatively new in the study of risk perception. Mental models focus on the way in which people conceptualize hazardous processes and allow researchers to pit a normative analysis (expert mental models) against a descriptive analysis (consumer mental models). Expert models were elicited by means of a three-wave Delphi procedure from 24 international experts, and consumer models were elicited from in-depth interviews with 25 Danish consumers. Consumers risk and benefit perceptions were subsequently validated in a national questionnaire survey with 800 Danish consumers.

Results and discussion: The results revealed that consumers' and experts' mental models differed as regards scope. Experts often focused on the types of hazards for which risk assessments can be conducted under current legal frameworks, whereas consumers were concerned about issues outside the scope of current legislation. Experts defined risk and benefit in terms of detailed chains of cause–effect relationships, whereas consumers used abstract concepts when reasoning about biological processes.

Conclusions: Several misconceptions became apparent in the consumer data as consumers overestimated both the positive effects of the functional food ingredient and negative side-effects. Another misconception was found as consumers perceived the functional food ingredient to have other health beneficial effects than those communicated by the interviewer. Half of the respondents wrongly assumed the functional food ingredient to have a weight-reducing effect, stating that it would be beneficial in connection to obesity control. The consumers who believed the functional food ingredient to be hazardous for humans also perceived it to be an "emergency solution" or an unnatural way of slimming. The focus on fat became evident when consumers were prompted with a card (initially extracted from the expert mental models) stating fat-soluble vitamins, as a majority of respondents evaluated fat-soluble vitamins as beneficial. Consumers wrongly assumed that the vitamin was part of Phytosterol and could dissolve fat. In general, the study revealed that the functional food ingredient investigated in this study was subject to many misunderstandings and misinterpretations, which indicate that consumers do not have a basic understanding of how nutrients influence their health.

Postmenopausal women and heart disease: acceptability of functional foods as a preventive measure

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Objective: This study focuses on postmenopausal women's view on heart disease among women and the potential acceptability of functional foods as a means of prevention.

Methodology: This study reports the results of eight focus group interviews with postmenopausal women (50–59 years old, $n = 73$) in Denmark and the UK.

Results and discussion: Although heart disease was recognized as a serious health problem and its occurrence among women recognized, it was predominantly conceived as a health problem affecting men and as being largely due to the relatively unhealthy lifestyles of men. Only a very small minority of participants were aware of the contribution of menopause to heart disease among women. Functional foods were generally not conceived as a means of prevention that is commensurate with the serious character of heart disease. It emerged from ranking tasks that functional foods do not constitute a product category as such. They occupy an anomalous position between "food" on the one hand and "medicine" on the other. Participants tend to dislike the idea of a "personal" food not intended to comprise part of a shared meal in the household, and also tend to distrust health claims promoted by the food industry as compared to the medical industry.

Build Your Food

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Objective: Build Your Food is an interdisciplinary project running for 4 years from September 2005. The project will generate fundamental and applicable knowledge on health-promoting polysaccharides and their functionality and implementation as matrices for aroma and bioactive substances aiming at understanding the basis for healthy and culinary foods.

Methodology: In the scientific literature there are ample nutritional experiments on foods that support the beneficial effect of polymeric carbohydrates such as β-glucan on sugar and cholesterol metabolism, digestion and the absorption of carcinogenic substances. However, owing to the complexity of the diets, the specific affinity effect on the molecular level can only be modelled by spectroscopy, molecular modelling and chemometrics and tested in less complex systems. This project will therefore focus on the molecular interactions of β-glucans compared with other fibre polymers and perform the necessary validation by chemical absorption studies and in an *in vitro* model of the digestive system.

Aroma release from a hydrocolloid matrix studying aroma model compounds can be quantitatively related to their sensory release profiles and even to the physiological sensing process itself. Such research requires molecular modelling, multivariate data analysis and sensory analysis to be combined, and the possible outcome will be new knowledge on the molecular affinities and mechanisms by which β-glucans function, including the mechanisms that guide the release of aroma from the food matrix, which is an important aspect of culinary quality.

In the pharmacological industry quantitative structure activity relationships (QSAR) are a very attractive technology used to screen for possible new active compounds. Similar methods can be used with advantage for the investigation of functionalities of food ingredients, in which, for example, molecular descriptors and molecular spectra of

different β-glucan preparations can be quantitatively related to their nutritional functionality. This project will introduce structure–function relationships to food systems in co-operation between four institutes and two industries representing the integrated food production and utilization chain. The aim is to demonstrate the potential in designing functional foods to be as attractive to the food industry as they are to the medical industry.

The poster will describe the project and the initial results.

Quantitative risk assessment strategies for novel foods (NOFORISK): an EU research project

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Objective: The NOFORISK project is a Specific Target Research Project (STREP) funded by the EU under the Sixth Framework Programme. NOFORISK develops and validate new methodologies for assessing the safety, nutritional properties and efficacy for health promotion of different types of second generation novel foods.

Methodology: The novel foods investigated in the project include (i) a genetically modified potato with altered levels and balance of inherent toxicants (glycoalkaloids); (ii) conventionally bred mutated rice with lowered levels of an antinutrient (phytic acid); and (iii) two functional food ingredients of natural origin (phytosterol esters). The project exploits genomic and non-targeted profiling techniques for characterizing these foods. Omic's techniques are also used to develop sensitive and specific biomarkers for *in vivo* and *in vitro* studies. Together, these will form the basis of NOFORISK's probabilistic approach to hazard characterization and nutritional benefit characterization.

Results and discussion: To test the genetically modified (GM) potato with altered glycoalkaloid (α-solanine and α-chaconine) levels, a preliminary study in hamsters was carried out to estimate the toxicity of the pure glycoalkaloids. In addition to the examination of toxicity parameters, liver and gut samples were taken from these animals for gene expression profiling and metabolite profiling. Results from the profiling will be used in the hazard assessment and for refinement of the study design when testing the whole GM potato in a 90 day hamster study. Phytosterol and phytostanol esters were tested in the Watanabe rabbit model, which is suitable for the detection of atherosclerosis. After exposure of the sterols and stanols through the feed for 19–120 days, samples were taken from the rabbits to examine the feasibility of transcriptomics and metabolomics together with *in vitro* cell culturing for the hazard assessment.

Conclusions: NOFORISK aims to deliver a comprehensive methodology for the combined quantitative assessment of risk and benefit of novel and functional foods based on probabilistic modelling of biological and exposure data.

Poster session, 15 March 2006

Submitted abstracts selected for the poster session, sorted by main author

Andersen JB. The pig as a model for human hepatic fat metabolism

Argyri K. Effect of milk peptides on iron solubility

Ashtiani FZ. The isomerization kinetics of lactose to lactulose in the presence of sodium hydroxide at constant pH Brindza J. Non-traditional fruit species: potential resources of functional food

Brindza P. Cornelian cherry (*Cornus mas* L.) in traditional and modern nutrition and medicine

Brix S. Effects of dietary fatty acids on T-cell responses induced by dendritic cells

Bruun S. Application of Fourier transform infrared and near-infrared spectroscopy in characterization of ligand-induced conformation changes in folate-binding protein

Budek AZ. Differential effects of casein-, whey- and soya-based milk diets on bone in newborn pigs

Csanádi J. The effect of somatic cell count on the texture properties of yoghurt from sheep's milk

Duan Zhi. Interactions between macromolecules and starter cultures in the cheese curd using phage display technique and bioimaging

Ertbjerg P. Total and heat-soluble collagen contents in different European cattle breeds: preliminary results

Gori K. Metabolic and regulatory changes in *Debaryomyces hansenii* upon exposure to increased sodium chloride concentrations

Hansen M. Genotoxicity of fructose, glucose and sucrose in the Big Blue® rat colon

Hedemann MS. *In vivo* measurement of the thickness of the mucus layer in the gastrointestinal tract: a rat model

Hellgren LI. The dietary fatty acid source regulates the activity of secretory sphingomyelinase, an enzyme involved in inflammatory signalling and atherogenesis

Hoac T. Selenium and selenoproteins in milk and mammary tissue

Janicke B. Differential effects of ferulic acid and *para*-coumaric acid on proliferation, S-phase distribution and length of S phase in the human colonic cell line Caco-2

Jazayery A. Effects of a low-calorie diet on serum leptin level and body fat distribution in obese men

Jørgensen ALW. Direct-affinity reverse extraction screening for bioactive food-derived peptides

Karlshøj K. Prediction of food safety by detection of volatile biomarkers by electronic nose technologies

Kiani A. Higher energy expenditure in nutritionally programmed offspring in later life in sheep

Kiskini A. Sensory characteristics and *in vitro* evaluation of iron dialysability of a gluten-free bread fortified with iron

Kjær TMR. Docosahexaenoic acid modulates lipid raft phospholipid composition and major histocompatibility complex class II expression in dendritic cells

Kjær TMR. Maternal dietary long-chain n-3 polyunsaturated fatty acid during gestation and lactation reduces the specific antibody response in neonatal mice

Michaelsen KF. Early determinants of body composition in 17-year-old adolescents from a Danish cohort of healthy term infants

Mølgaard C. The effect of seven day supplementation with milk protein fractions and milk-minerals on insulin-like growth factors and glucose–insulin metabolism in Danish prepubertal boys.

Mu H. Investigating the effect of n-3 dietary fatty acids on liver phospholipid composition by liquid chromatography–mass spectrometry

Nielsen A. Control and freedom: going shopping with weight losers.

Nørgaard MK. Children's influence on family decision making in food buying and consumption with a focus on healthy food products

Önning G. Health effects of foods enriched with β-glucans from cereals

Poulsen L. Bioavailability and metabolism of hesperetin and naringenin from enzymically or non-enzymically treated orange juice

Pripp AH. Data mining on the relationship between health-related responses and taste of bioactive peptides derived from food proteins

Raff M. Conjugated linoleic acid and vaccenic acid have no effect on blood pressure and isobar arterial elasticity in healthy young men

Ravn-Haren G. Interaction between alcohol intake and oxidative defence in breast cancer

Ravn-Haren G. Effect of long-term selenium yeast supplementation on biomarkers of oxidative defence in healthy elderly volunteers

Sangild PT. Casein-stimulated bone growth and whey-stimulated tissue growth in newborn immunocompromised pigs

Siggers JL. Amniotic fluid decreases the incidence of necrotizing enterocolitis in preterm pigs

Siggers RH. Mode of delivery affects diet-induced small intestine atrophy and dysfunction in preterm pigs

Ueland Ø. Perceived importance of healthiness in meals

Vilkauskaite G. Tomato consumption in Lithuania

Young JF. Myotubes as a model for studying the effects of creatine, analysis of metabolites and protein regulation

Young JF. Human colon cells show biphasic survival characteristics in response to increasing falcarinol concentrations

The pig as a model for human hepatic fat metabolism

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Objective: The pig, as a animal model for humans, provides an excellent possibility to examine the importance of nutrition in relation to malfunction of hepatic fat and carbohydrate metabolism (e.g. hepatic steatosis). The advantage of using the common domesticated pig (e.g. Landrace or Yorkshire) lies is the possibilities of taking consecutive blood samples and repeated liver biopsies so that the animal can serve as its own control. Having focus on animal welfare and working with intact/non-compromised animals, only small liver biopsies should be taken. Consequently, methods are needed for obtaining small liver biopsies on living pigs and subsequently quantifying fat metabolism on these biopsies.

Methodology: The following methods will be presented: (i) use of ultrasound for localization of the liver before percutaneous sampling of small biopsies with a True-Cut biopsy needle (one biopsy = ~20 mg); (ii) analysis of liver tissue content of triacylglycerol (TAG), glycogen and total protein using small biopsies and an autoanalyser; and (ii) analysis of the capacity for complete oxidation of [1-^{14}C]long-chain fatty acids (LCFA) in liver tissue, and the capacity for ketogenesis of [1-^{14}C]LCFA.

Results and discussion: Ultrasound is needed for detection of where the biopsies can be taken, but also for measuring the depth of the penetration necessary for obtaining the biopsy (length of the biopsy needle). Here it is important to use an ultrasound probe with sufficient power to penetrate more than 20 cm of skin, subcutaneous muscle and fat layers and liver tissue. As an alternative to traditional methods (using a minimum of 80–150 mg of tissue), a method has been developed for TAG, glycogen and total protein analysis of liver tissue (18–50 mg) having a CV between 5 and 9% on repeated samples from the same liver. With no further preparation, liver biopsies (12–25 mg of tissue) can be used for measuring the *in vitro* capacity of LCFA metabolism. The *in vitro* methods are affected by incubation time (linear increase between 0.5 and 1.0 h) and different types of medium (e.g. linear increase between 0.5 and 1.0 mM palmitate), showing a physiological response.

Conclusions: It is possible to obtain liver tissue using ultrasound and a True-Cut biopsy instrument working with live pigs. Further, these small liver biopsies can be used for measuring chemical content and capacity of LCFA oxidation.

Effect of milk peptides on iron solubility

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Objective: The overall objective of this study was to identify peptides in cow's milk that enhance iron bioavailability. The

specific objective of this study was to test iron solubility in the presence of milk peptides, obtained from milk digested *in vitro* and fractionated by gel filtration and high-performance liquid chromatography (HPLC).

Methodology: Cow's milk, 0% fat, was subjected to *in vitro* digestion (Kapsokefalou and Miller, J Food Sci 1991; 56:352). This procedure simulates the gastrointestinal process by subjecting samples to a 4.5 h incubation at 37°C, at different pH, in the presence of peptic enzymes and a dialysis bag, allowing the separation of a low molecular weight (<6000) soluble fraction. The soluble, low molecular weight digest was further fractionated by gel filtration and subsequently by HPLC. All fractions eluted, as well as isolated peptides obtained commercially, were incubated with ferric chloride. The solubility of iron was determined spectrophotometrically with ferrozine.

Results and discussion: The most important finding was that fractions of milk digests obtained through gel filtration and HPLC had a significant effect on iron solubility compared with the effect of non-fractionated milk digests. Some fractions enhanced iron solubility while others had no effect. The peptides that were obtained commercially enhanced iron solubility in a dose–response manner. However, their effect was lower than that of the fractions obtained by gel filtration or HPLC. The solubility of iron under conditions that simulate the gastrointestinal environment could be used as an index of iron bioavailability. It is of interest to investigate the potential beneficial effect of milk peptides, particularly because selected milk peptides have been recently associated with bioactivity in various physiological functions (Philanto-Leppala, in Encyclopaedia of Dairy Sciences; 2003). Peptides of milk origin are considered as highly prominent ingredients for health-promoting, functional foods. These results encourage further research on the properties of milk peptides on iron bioavailability.

Conclusions: Selected milk peptides may enhance iron solubility. In this study milk peptides that exhibited an enhancing effect on iron solubility were formed during the *in vitro* digestion of milk and isolated by gel filtration and HPLC.

The isomerization kinetics of lactose to lactulose in the presence of sodium hydroxide at constant pH

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Objective: Lactulose is a synthetic ketose disaccharide that is not found naturally. Lactulose has been used extensively for treatment of portal systemic encephalopathy and chronic constipation. It is also used as a *Bifidus* factor in nutrition and is known to be a very important humanizing factor in infant formula, and is added to commercial infant formula products and various milk products. This sugar has greater sweetness and solubility than lactose and if produced economically, can be used widely in baking and confectionery applications. The aim of this work is to introduce a suitable reaction model for isomerization of lactose to lactulose with sodium hydroxide at constant pH in order to find a beneficial tool for predicting the trend of reaction in different conditions and study the effect of important parameters such as pH and temperature on the reaction.

Methodology: After finding a suitable model for reaction and solving the rate equations, equations for concentration of each component were determined. The experiments were carried out at three temperatures (50, 60 and 70°C) and three pH levels (10, 10.5 and 11). Samples were taken at different times. The content of different sugars was determined by high-performance liquid chromatography (HPLC). The data obtained from HPLC were used to calculate rate constants.

Results and discussion: During the isomerization of lactose to lactulose using sodium hydroxide as a catalyst, a high level of degradation occurred; therefore, to decrease byproduct formation, it is better to use the minimum catalyst concentration. The highest yield of lactulose production occurred at the highest possible temperature. The kinetic equations of lactulose formation and lactose degradation in an alkaline medium were also determined.

Conclusions: A mathematical kinetics model was developed. By studying the kinetics of lactose isomerization to lactulose and the relationship between rate constants and temperature and pH, the optimum conditions for this reaction were obtained. This tool can be used to predict the reaction trend in different conditions and these kinetic equations can be used for reaction simulation.

Non-traditional fruit species: potential resources of functional food

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Objective: The aims were monitoring of neglected plant species in Slovakia, economic evaluation of the genotype traits, nutritional value determination for fruits and food, and characterization of the fruit and food microflora.

Methodology: Morphometric and chemical studies conducted in 2003–2005 evaluated fruits of 100 genotypes of black mulberry (*Morus nigra* L.) and 130 genotypes of true service tree (*Sorbus domestica* L.) representing the populations occurring on the Slovak territory. From the morphometric analysis of fruit, of all genotypes, economically important traits were registered, together with the chemical determination of saccharides, lipids, vitamins, proteins and other components. The microflora composition on the fruits, food and different plant parts was identified.

Results and discussion: This experimental study of 100 black mulberry genotypes showed interesting differences in fruit weight (15–127 g), vitamin C content (2.26–18.9 mg 100 g^{-1}), total saccharides (4.7–19.7 mg kg^{-1}) and lipid

content (1.9%). The following values were found: lipids in seeds 16%, in fruit linoleic acid represented 54.7% of lipid content, and the pigment content in fruit was 2.1–6.3 mg kg^{-1}. Significant differences also occurred with the true service tree. The fruit weight ranged from 2.9 to 36.1 g, vitamin C content from 80 to 120 mg 100 g^{-1}, vitamin A from 10 to 15 mg 100 g^{-1}, lipids in seeds 20%, linoleic acid in seeds 24.3% and pectins in fruit 1.3–2.0%. Similarly important differences were seen with the macronutrients and micronutrients. The results showed an economically based argument for using selected genotypes of these species for practical purpose in nutrition, pharmacology and cosmetics, and for landscaping. At present, fruit are processed using both traditional and modern technologies. It is historically documented that different plant parts were used not for consumption but for medicinal aims. This trend should be revived as shown in the literature where there are available references for doing so.

Conclusions: It was experimentally documented that the black mulberry (*Morus nigra* L.) and true service tree (*Sorbus domestica* L.) represent important resources of valuable fruit, which can be used as alternative species for nutrition, pharmaceutics and cosmetics, and moreover as resources for the extraction of biologically active substances suitable for the production of functional food.

Cornelian cherry (*Cornus mas* L.) in traditional and modern nutrition and medicine

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Objective: The aims were to collect information on the traditional use of the cornelian cherry (*Cornus mas* L.) in nutrition and medicine, to determine the economic value of the cornelian cherry population in Slovakia, and to perform chemical analyses of fruits.

Methodology: The study evaluated 134 ecotypes forming populations in the Gemer region of southern Slovakia. Fifty ripe fruits from each ecotype were tested and several traits evaluated, including weight (g), length, width and thickness (mm). The content of macroelements and microelements, proteins, lipids and vitamins was determined in lyophilized fruit flesh. Knowledge about the traditional uses of fruits in nutrition and medicine was obtained from local inhabitants and old literature sources.

Results and discussion: In the experimental pool, the range of fruit weight was 0.5–3.4 g, fruit length 12.0–19.5 mm and fruit thickness 7.4–15.2 mm. Among evaluated genotypes there were also significant differences in other traits. Genotypes were selected from the evaluated collection for practical use. Cornelian cherry shrubs and trees grow up to altitude of 1400 m. Cornelian cherry does not have difficult growing conditions; it grows very well on sunny sites, and tolerates drought and calciferous soils. Shrubs or formed trees are characterized by their hard wood and can live for up to 200–300 years. *Cornus mas* tolerates frost to $-40^{\circ}C$. Shrubs of the cornelian cherry provide economically valuable fruit, which ripen from August to the beginning of September. Based on the chemical analyses, in the collection of tested genotypes the vitamin C content was in the range 16.4–38.5 mg 100 g^{-1}, total sugars 6.5–15.1%, organic acids 4.6–7.4%, pH 2.7–3.2, lipids in the stone 4.6%, lipids in the flesh 0.3%, content of linoleic acid in the stone 67.3% and in the flesh 36.54%. The study of traditional knowledge and experiences of the use of the cornelian cherry, revealed that fruits of the cornelian cherry have been used by Slovaks for centuries in both the fresh and dry states. Fruits were also preserved into compotes, jams, syrups and liqueurs, and used in traditional medicine for healing various diseases.

Conclusions: This study evaluated morphometric traits of the fruit and stones of the cornelian cherry, and the chemical characteristics of fruit flesh. Significant variability was found in the shape and dimensions of the fruit and stones. The chemical analyses showed important differences in fruit composition among ecotypes. Fruit flesh of cornelian cherry has high nutritional values, including biologically active substances suitable for human nutrition.

Effects of dietary fatty acids on T-cell responses induced by dendritic cells

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Objective: Dietary fatty acids, especially n-3 polyunsaturated fatty acids (PUFAs), have been shown to affect T-cell responses, and incorporation of n-3 PUFAs in the T-cell membrane may therefore influence the tendency of T-cells to respond following dendritic cell (DC) interaction.

Methodology: The study examined the effect of feeding diets rich in n-3 PUFAs, n-6 PUFAs or saturated fatty acids on the response induced in murine $CD4^{+}$ T-cells by bone marrow-derived DCs stimulated with different commensal bacteria.

Results and discussion: DC-induced T-cell proliferation revealed that activation of T-cells was profoundly affected by the fatty acid composition of the cell membrane, giving rise to a markedly reduced T-cell activation when n-3 PUFAs were incorporated into the T-cell membrane.

Conclusions: The effectiveness of DC signalling to T-cells seems to be highly dependent on the fatty acid composition of the T-cell membrane, highlighting the importance of dietary control of the immune system.

Application of Fourier transform infrared and near-infrared spectroscopy in characterization of ligand-induced conformation changes in folate-binding protein

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Objective: Folate-binding proteins (FBPs) are involved in, among other things, the distribution, excretion and homo-

eostasis of folate in the body, and soluble forms of FBP exist in most body fluids. Folate binding at pH 5.0 is associated with drastic conformational changes in FBP, as revealed by CD studies. At the same pH, the ligand binding is highly dependent on the buffer type; binding takes place in formate and phosphate buffer, but not in acetate and citrate buffers at low FBP concentrations, meaning that these metabolites could influence folate homoeostasis in acidic intracellular environments. This study examines the secondary structure of bovine milk FBP before and after ligation in several buffer types of pH 5.0. The effect of pH is also examined.

Methodology: Fourier transform infrared spectroscopy (FTIR) and Fourier transform near-infrared spectroscopy (FT-NIR) were used to measure FBP at high protein concentrations.

Results and discussion: FTIR and FT-NIR spectra showed a β-sheet to α-helix transition upon ligation in acetate and phosphate buffers of pH 5.0. The formation of intermolecular β-sheets was also indicated from the FTIR spectra in accordance with a dimerization taking place at pH 5.0. The buffer-type effect at low FBP concentrations was supported by the FT-NIR and FTIR spectra showing distinct conformations of unligated FBP in formate and phosphate buffers compared with acetate and citrate buffers. Intense amino acid side-chain absorption at 2260 nm (in the NIR) in the case of formate and phosphate buffers suggests a different quaternary structure or solvation state of FBP in these buffers. The spectral distinction diminished upon ligation in phosphate buffer, but not in formate buffer, as FBP in formate (and citrate) only showed small and non-significant spectral changes. The FT-NIR spectra of FBP in phosphate buffer of pH 7.4 revealed contradictory effects on the side-chains compared with the pH 5.0 studies, reflecting the different polymerization events at the two pH values.

Conclusions: FTIR showed secondary structure changes upon ligation in acetate and phosphate buffers, while FT-NIR also indicated other rearrangements concerning the amino acid side-chains.

Differential effects of casein-, whey- and soya-based milk diets on bone in newborn pigs

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Objective: Infancy is an important period for skeletal growth and formula-feeding is extensively used during this time. The aim was to investigate the effects of proteins from infant formulae on bone development in newborn pigs.

Methodology: Nineteen term piglets were fed milk diets (15 ml kg^{-1} 1½ h^{-1}) by orogastric tubes. They were randomly assigned to three groups with different protein sources [casein (C) $n=5$; whey (W) $n=9$; soya (S) $n=5$], but with equal content of fat (7.6%), total protein (5.5%), lactose (5.3%), calcium (0.19%) and phosphorus (0.14%). After 7 days the pigs were killed. Fasting blood was collected and serum analysed for insulin-like growth factor-1 (IGF-1) and insulin. The femora and tibiae were dissected, and volumetric bone mineral content (BMC, mg mm^{-1}), bone mineral density (BMD, mg cm^{-3}) and geometric parameters of cortical bone were measured by quantitative computed tomography. Bone mechanical strength was measured by a three-point bending test.

Results and discussion: During the study, the average group weight increased by 8.8% for C, and decreased by 4.6% and 4.8% for W and S, respectively ($p=0.03$). After 7 days trabecular BMD at the femoral neck (FN) was lower in W (LSmeans 319.6) compared with C (LSmeans 351.0) and S (LSmeans 363.2) ($p=0.02$). Trabecular BMC at FN was higher in C (LSmeans 9.0) compared with S (LSmeans 6.8) but not with W (LSmeans 7.9) ($p=0.02$). At the proximal tibia W had lower trabecular BMD (LSmeans 269.2) compared with C (LSmeans 305.5) and S (LSmeans 296.6) ($p=0.01$). The endocortical circumference of midshaft tibia tended to be smaller in C ($p=0.053$). Cortical BMD and BMC were not different between the groups at any sites (all $p>0.1$). There were no significant changes in bone mechanical strength ($p=0.4$). End-time IGF-1 was not different between the groups ($p=0.6$). Most of serum insulin samples were below the detection limit.

Conclusions: The pigs fed C already had improved bone quality at trabecular sites after 7 days. Similar effects of milk casein may be present in newborn infants. Further studies are needed, with longer duration and larger sample size.

The effect of somatic cell count on the texture properties of yoghurt from sheep's milk

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Objective: Somatic cell count (SCC) is a good indicator of the health of milking sheep, which denotes the rate of mastitis. Current knowledge is unsatisfactory and insufficient regarding the occurrence grade of sheep's milk from an infected udder, and mostly about the unfavourable effects of high SCC on dairy processing regarding the negative change in texture. The aim was to collect information about the influence of SCC of Hungarian sheep milk on the acid milk gel.

Methodology: To characterize the substance properties of yoghurt made from sheep's milk, the study investigated whey leakage (Al-Khajafi et al., 1977) and some other parameters that could be measured by the Stevenson QTS-25 instrument (CNS Farnell, UK), i.e. hardness, adhesive force and adhesiveness.

Results: This investigation of the effects of the SCC of sheep's milk on the texture of the a fermented product (yoghurt), showed that the effects of the SCC were similar to and verified according to the literature data on cow's milk. Regarding whey leakage, in cases <1 million cells cm^{-3} no major deviation can be expected. There was very close

correlation ($r^2 = 0.931$) between the SCC and whey leakage. Regarding the effects on texture, similar results to the case of whey leakage were obtained. There is a non-proven correlation up to 800 000 cells cm^{-3} concerning the negative effect of the SCC. Above 800 000 cells cm^{-3} (in the case of 1 million cells cm^{-3} for the adhesive force the correlation was very close; for example, in the case of hardness $r^2 = 0.763$; adhesive force $r^2 = 0.816$).

Conclusions: The high SCC in ewe's milk spoils the substance properties of the acid gel. The devaluation effect is not sufficient below an SCC of 800 000 cm^{-3}. A value of 800 000 cm^{-3} SCC is suggested in the development of the raw sheep's milk qualification system and the quality of sheep milk. This value could be achieved without too much difficulty using modern farming systems. The authors propose considering this value as a limit in the development of the Hungarian quality qualification system in the future.

Interactions between macromolecules and starter cultures in cheese curd using phage display technique and bioimaging

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Objective: The aim of this project is, by using an antibody phage display technique, to generate antibody fragments against selected structures of cheese milk, primarily specific casein fractions and peptidases from LAB. The casein fractions should traverse the cleavage site of enzymes responsible for proteolysis during cheese ripening. So long as the cleavage site is intact, the antibody will bind to the casein. However, after cleavage of the peptide bond by the enzymes, the antibody will no longer be able to recognize the substrate. The antibody fragments will be labelled by fusion with different fluorescent proteins. Bioimaging techniques can then be used to follow the selected macromolecules of cheese during ripening. Simultaneously, the location of single cells and discrete microcolonies will be determined.

Methodology: Antibody phage display is a molecular technique by which antibody fragments (scFv, single-chain variable antibody fragment) can be expressed at the surface of bacteriophage particles (usually M13 bacteriophage). Such phages thereby become vehicles for expression that not only carry within them the nucleotide sequence encoding expressed scFv, but also have the capacity to replicate. Populations of variant scFvs can be displayed on the phage, by which means an *in vitro* library of scFvs can be generated. Using this technique, immunization is bypassed and functional antibodies can be produced.

Results and discussion: The procedure for screening specific antibody phage against target antigen has been optimized. An anti-α-casein antibody phage sublibrary has been produced, which will be used for generating antibody phage against fractions from α s1- and α s2-casein. This sublibrary showed high enough affinity against α -casein. The selected fractions from α s1-casein (14 to 16mer peptides) have been synthesized. The method for immobilization of small peptides on immunosurfaces is under investigation.

Conclusions: It is expected that this project will provide unprecedented knowledge about the microscale changes in the cheese matrix during ripening, which may be developed to evaluate and predict the influence of processing parameters and starter cultures on the final food cheese quality.

Total and heat-soluble collagen contents in different European cattle breeds: preliminary results

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Objective: The aims of the study were to investigate the effect of breed on total and heat-soluble collagen in m. longissimus thoracis and to study the correlation between total collagen and compression data.

Methodology: In total, 437 bulls of 15 different breeds (Jersey, South Devon, Aberdeen Angus, Highland, Holstein, Danish Red Cattle, Simmental, Asturiana de los Valles, Casina, Avileña, Pirenaica, Piemontese, Marchigiana, Limousin and Charolais) were slaughtered at 15 months of age. Samples were collected from m. longissimus thoracis 24 h p.m. For determination of total and heat soluble collagen 6 g of meat was finely chopped, mixed with 0.9% NaCl and heat-treated for 2 h at 90°C. After homogenization and centrifugation (15 min at $4000 \times g$), both the supernatant and pellet were hydrolysed in 6.0 M HCl overnight in a sandbath at 110°C. The concentration of hydroxyproline was determined and a factor of 7.14 was used to convert hydroxyproline to collagen. The amount of soluble collagen was calculated from the hydroxyproline concentration in the supernatant, and the total collagen was calculated from the sum of the hydroxyproline concentration in the pellet and in the supernatant.

Results and discussion: Breed differences were observed in total and heat-soluble collagen. The dairy breeds (e.g. Jersey, Holstein) contained higher amounts of collagen than the meat specialized breeds. The meat specialized animals (e.g. Piemontese, Limousin) contained significantly less collagen than the other breeds and less heat-soluble collagen than most of them. For only a few of the 15 breeds (Asturiana de los Valles and Pirenaica, $r = 0.5–0.7$, $p < 0.01$) was there a high correlation between compression at 80% (C80) determined 48 h and 10 days p.m. and total collagen content.

Conclusions: Total and heat-soluble collagen varies between breeds. The correlation between total collagen and C80 is breed dependent.

Metabolic and regulatory changes in *Debaryomyces hansenii* upon exposure to increased sodium chloride concentrations

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Objective: The highly NaCl-tolerant yeast *Debaryomyces hansenii* is used as a starter culture for the production of surface-ripened cheeses and sausages. The growth of *D. hansenii* is important to eliminate growth of mould contaminants and pathogenic bacteria such as *Listeria monocytogenes*. The physiological properties of *D. hansenii* have been investigated to some extent; however, the genetic and proteomic knowledge on *D. hansenii* is insufficient. Recently, the genome of *D. hansenii* has been fully sequenced, and sequences are available at http://cbi.labri.fr/Genolevures/elt/DEHA. The aim of this study was to investigate the proteome of *D. hansenii* to obtain new knowledge of how this highly NaCl-tolerant yeast species deals with NaCl stress.

Methodology: The proteome of *Debaryomyces hansenii* var. *hansenii* (type strain CBS 767) was investigated by two-dimensional polyacrylamide gel electrophoresis (2D PAGE) and protein spots was identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS).

Results and discussion: The first proteomic map for *D. hansenii* was presented. The influence of NaCl on the proteome was investigated, resulting in identification of several proteins being either induced or repressed upon exposure to increased NaCl concentrations. Induced proteins were enzymes involved in glycerol synthesis and assimilation, upper part of glycolysis and heat shock proteins, whereas repressed proteins were involved in synthesis of amino acids and nucleotides.

Conclusions: The identification of the proteome of *D. hansenii* including induced and repressed proteins upon exposure to high NaCl concentrations will add to the understanding of how this highly NaCl-tolerant yeast species deals with NaCl stress.

Genotoxicity of fructose, glucose and sucrose in the Big Blue® rat colon

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Objective: The aims were investigate whether the colon genotoxicity of sucrose can be ascribed specifically to either fructose or glucose, and whether endogenous metabolism, colonic fermentation or insulin sensitivity is related to mutation.

Methodology: Groups of 11 male Big Blue® rats were fed with 30% sucrose, glucose or fructose as part of a semi-synthetic diet. The 13 controls were fed potato starch instead of sugars. Metabonomic analyses on plasma and urine samples were performed by ^{1}H-nuclear magnetic resonance and multivariate statistics. The mutation rates in the colon were analysed using a kit, and bulky DNA adducts by ^{32}P-postlabelling. The caecal concentration of acetate, propionate and buturate was determined by capillary zone electrophoresis. Plasma C-peptide was analysed using a kit.

Results and discussion: Glucose, fructose and sucrose increased mutation rates and adduct formation to a similar extent in the colon. These small carbohydrates also decreased the caecal concentration of acetate and propionate, indicating changes in the composition or the activity of the microflora. Decreased plasma acetate concentration and the composition of other metabolites confirmed this. The colon epithelium cells may be affected by decreased acetate concentration because it is an important substrate in the synthesis of their membranes. No changes were seen on C-peptide or other markers related to insulin resistance.

Conclusions: High sugar levels in the rat feed at the expense of potato starch led to increased genotoxicity and increased mutation rate in the colon. The mechanism may be related to changes in the colonic microflora or to changes in the substrate for the epithelial cells. Insulin resistance does not seem to be involved.

In vivo measurement of the thickness of the mucus layer in the gastrointestinal tract: a rat model

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Objective: The objective of using the model described in this paper is to measure the thickness of the mucus layer *in vivo*.

Methodology: In an ongoing experiment rats are fed six diets differing in content and composition of non-digestible carbohydrates. The basal diet is a semi-synthetic, non-fibre diet. To this diet is added one of five non-digestible carbohydrate sources (cellulose, pectin, inulin, resistant starch or barley hulls). The rats were fed the experimental diets for 4 weeks before measurement of the thickness of the mucus layer. Rats were anesthetized and the colon was exteriorized and opened along the antimesenteric border. The rat was place on a microscope stage and the intestine was draped over a truncated cone with the luminal side up. A mucosal chamber with a hole was placed over the exposed mucosa and the chamber was filled with 0.9% NaCl. The mucus gel was covered with carbon particles to visualize the

surface of the gel. A micropipette held by a micromanipulator was pushed into the mucus gel at an angle of $\sim 30^{\circ}$ to the cell surface. The distance from the luminal surface of the mucus layer to the epithelial cell surface of the mucosa was measured with a digimatic indicator. The procedure was carried out under observation through a stereomicroscope.

Results and discussion: The diets used in the present experiment were composed to vary with respect to physicochemical properties, fermentability and fermentation products in order to investigate the interactions between the mucus layer and dietary fibre. The thickness of the mucus layer measured varied between 70 and 700 μm; however, data still need to be analysed with respect to the effect of diet. The mucus layer covers the lining of the gastrointestinal tract from the stomach to the colon. It has an important role in protecting the epithelial cell layer against excessive mechanical stress, pathogens, digestive enzymes, etc., and is proposed to be a key factor in maintaining intestinal health. Measurement of the thickness of the mucus layer has been a challenge as the mucus layer is dissolved or shrinks during traditional fixation.

Conclusions: This model allows measurements of the mucus layer along the gastrointestinal tract *in vivo* and may become a valuable tool in assessing the health effects of prebiotics and probiotics in the gastrointestinal tract.

The dietary fatty acid source regulates the activity of secretory sphingomyelinase, an enzyme involved in inflammatory signalling and atherogenesis

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Objective: The secretory sphingomyelinase (sSMase) is secreted from endothelial and immune cells as a response to inflammatory signals. The enzyme hydrolyses the membrane phospholipid sphingomyelin to the signalling lipid ceramide. It has been showed that activation and secretion of sSMase is an essential initial step in the signalling cascade linking inflammatory elicitors, such as lipopolysaccharide, to the production of inflammatory cytokines (Bollinger et al., Biochim Biophys Acta 2005) and that increased activity of sSMase is a strong inducer of macrophagal foam-cell formation (Marathe et al. Arteriosclerosis, Thrombosis and Vascular Biology 2000;20:2607–13). Hence, regulation of sSMase activity could be important in the development of the metabolic syndrome. This study investigated whether the dietary fatty acid intake (butter vs grapeseed oil) alters the activity of sSMase in serum.

Methodology: Eight rats were fed a semi-synthetic diet containing 31 $E\%$ fat as either butter or grapeseed oil (70% polyunsaturated) for 12 weeks before being killed by heart puncture. Serum was isolated and the sSMase activity was determined.

Results and discussion: The serum activity of sSMase was 241 ± 57 fmol ml serum^{-1} h^{-1} in the butter-fed rats and 373 ± 50 mol ml serum^{-1} h^{-1} in the rats fed grapeseed oil. Thus, butter intake seems to have an advantageous effect on the secretion of sSMase. This may seem to be in contradiction to the present view of butter as a highly atherogenic product. However, the ratio of n-6/n-3 polyunsaturated fatty acids in serum lipids in the butter-fed rats was 6.7 ± 0.7, while it was 65 ± 14 in the grapeseed-oil fed rats. Hence, the fatty acid profile was proinflammatory in the grapeseed oil-fed, compared with the butter-fed rats. One hypothesis is that the increased n-6/n-3 PUFA ratio stimulates secretion of sSMase, and that this partly explains the anti-inflammatory properties of n-3 fatty acids.

Conclusion: Intake of butter reduces the activity of the sSMase, compared with intake of a highly unsaturated vegetable oil.

Selenium and selenoproteins in milk and mammary tissue

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Objective: The aims were to investigate the effect of selenium (Se) supplementation in cow feed on Se and other trace elements in bovine milk, and to study the relationships between Se and selenoproteins in mammary tissue.

Methodology: Cows were fed with either Se-fortified feed (25 mg organic Se day^{-1}) or control feed. Selenium content in bovine blood plasma and milk was measured with graphite furnace atomic absorption spectrometry (GF-AAS) and inductively coupled plasma mass spectrometry (ICP-MS), respectively. Size-exclusion chromatography coupled to ICP-MS was used for separation and detection of Se, Fe, Cu and Zn compounds in whey and plasma. In another project mammary tissue obtained at slaughter was analysed for Se content (ICP-MS) and the enzyme activities of thioredoxin reductase (TR) and glutathione peroxidase (GSHPx).

Results and discussion: The Se content in milk from cows given supplements of Se-enriched yeast at 25 mg day^{-1} increased more than six-fold compared with that in the control group, while the increase in plasma was only three-fold. The increase in Se content in whey and plasma was associated with several protein fractions and the increases were more obvious in whey than in plasma. The distribution pattern of Fe, Cu and Zn in whey before Se supplementation was similar to that after Se supplementation and this similarity was also observed in plasma. In mammary tissue, the activity of TR varied six-fold between tissue samples, and the corresponding figure for GSHPx was 15-fold. There were positive correlations between the following variables: Se and TR ($p < 0.01$), TR and GSHPx ($p < 0.01$) and Se and protein content ($p < 0.01$). This indicated that Se status regulated selenoprotein activities in bovine mammary gland in some extent, but other variables also seemed to be important.

Conclusions: The Se content in milk increased more rapidly and to a larger extent than in plasma from cows given Se supplementation and this increase was found to be associated with several protein fractions. The distribution of Fe, Cu and Zn in whey and blood plasma was not affected by Se supplementation of cow feed. The activities of TR and GSHPx in mammary tissue varied widely, and there were positive correlations between Se and TR, TR and GSHPx, and Se and protein content in bovine mammary tissue.

Differential effects of ferulic acid and *para*-coumaric acid on proliferation, S-phase distribution and length of S phase in the human colonic cell line Caco-2

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Objective: A high intake of wholegrain cereals which contain ferulic acid (FA) and *p*-coumaric acid (*p*-CA) and other bioactive compounds is associated with health benefits. Several mechanisms of action have been proposed, but their relative importance is unclear. These include antioxidative and anticarcinogenic effects, lowering of low-density lipoprotein cholesterol and effects on the cell cycle. The aim of the study was to investigate the molecular mechanisms of action of FA and *p*-CA in the Caco-2 cell line.

Methodology: Cell metabolic activity was assessed by the MTT and Alamar blue assays. Cell cycle phase distribution and cell cycle kinetics were measured by flow cytometric analysis.

Results and discussion: Addition of FA up to 150 μmol l^{-1} for 1–3 days did not diminish cell metabolic activity significantly, but at 1500 μmol l^{-1} a small decrease in metabolic activity was observed. *p*-CA did not show any effects on metabolic activity up to 1500 μmol l^{-1}. However, both compounds at 1500 μmol l^{-1} decreased the number of cells to 54–75% of control. Both FA and *p*-CA also decreased the proportion of cells in the G_1 phase and increased the proportion of cells in the S and G_2 phases. Treatment with FA significantly increased the length of the S phase, whereas *p*-CA did not.

Conclusions: FA and *p*-CA inhibited cell proliferation, presumably by affecting different cell-cycle phases. Calculations showed that the concentrations of FA and *p*-CA used may be possible to attain in the intestinal lumen.

Effects of a low-calorie diet on serum leptin level and body fat distribution in obese men

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Objective: The aim was to determine the effect of a 1200 kcal diet on anthropometric parameters, body fat distribution and serum leptin concentration in obese men.

Methodology: One-hundred obese men, 25.8 ± 6.0 (18–43) years old, body mass index (BMI) = 30–36, matched for general characteristics, were put on a 1200 kcal diet (energy shares of fat, protein and carbohydrate 25%, 15% and 60%, respectively) for 8–16 weeks. BMI, waist–hip ratio (WHR), and fasting serum leptin and insulin levels were determined at the beginning and end of the period. Energy and nutrient intakes were calculated from two 3 day food records during the study.

Results and discussion: The 1200 kcal diet brought about a statistically significant ($p = 001$) 7% reduction in body weight and BMI. The reduction in WHR, although significant, was only 2%. These data indicate that the diet affected primarily the total fat loss, rather than the fat distribution. There were statistically significant associations between energy intake or percentage dietary energy from fat and leptin (as well as BMI and WHR). However, when percentage dietary energy from fat was corrected for BMI, the association disappeared. The reductions in the serum levels of leptin (35.6%) and insulin (32.0%) were quite large and statistically significant ($p = 0.01$). Further analysis of the data showed the only factor causing a reduction in leptin concentration was percentage weight loss ($R^2 = 0.46$; $\beta = 0.167 \pm 0.084$, $p = 0.04$), which confirms some of the previous findings reported in the literature. There was no correlation between leptin and insulin before weight loss, whereas after weight loss a significant positive correlation appeared. This indicates that the serum levels of these two hormones do not increase in the same proportion in obesity.

Conclusions: A low-calorie diet can bring about desirable metabolic changes with regard to such hormones as leptin and insulin accompanying weight loss in obese men, but with no appreciable change in the body fat distribution.

Direct-affinity reverse extraction screening for bioactive food-derived peptides

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Foods of the future should not only provide appropriate nutrients and sufficient energy for human function, but also contribute to the well-being and health of consumers. Bioactive milk-derived peptides occur in the highest concentrations in the gastrointestinal tract, where innate immune responses mediated by Toll-like receptors (TLR) are particularly important for the host defence against microbial infection. Weaning of piglets dramatically affects the ability of lymphocytes to respond to bacterial lipopolysaccharide mediated by TLR-4; thus, bioactive constituents of milk could potentially affect the TLR-mediated response in the gastrointestinal tract. To characterize novel foods for presence of potentially bioactive components, rapidly and efficiently, it is proposed

that bioactive peptides could be identified by direct affinity reverse extraction (DARE), arguing that for a peptide to have a biological effect it must bind to a relevant target protein with high affinity. This novel method uses immobilized target proteins to extract peptides with high binding affinity from complex hydrolysates containing thousands of different peptides. TLRs and other relevant proteins will be used as targets directly immobilized on matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) plates for subsequent identification by accurate mass analysis and sequencing of bound peptides.

Prediction of food safety by detection of volatile biomarkers by electronic nose technologies

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Objective: Headspace analysis by electronic nose (e-nose) measurement has a potential in indirect determination of spoilage of food, more specifically production of mycotoxins by food spoilage fungi. This is an introduction to how the e-nose works, and the potential of such e-nose technologies compared with traditional methods such as GC-MS analysis and LC-MS analysis. To illustrate the potential of this method an example is illustrated in the following sections.

Methodology: A total of 20 isolates of the cheese-associated species *Geotrichum candidum*, *Penicillium camemberti*, *P. nordicum* and *P. roqueforti* and its closely related species *P. paneum*, *P. carneum*, as well as the non-cheese-associated *P. expansum* were investigated. The isolates were inoculated, in four replicates, on yeast extract sucrose agar in 20 ml headspace flasks and e-nose analysis was performed daily for a 7 day period. The results obtained from the e-nose analysis were correlated with analysis of mycotoxin content, which was done by liquid chromatography–mass spectrometry (LC-MS) analysis.

Results and discussion: After principal component analysis (PCA) was performed on the e-nose readings it was evident that the trend went from no separation of the species, neither from each another nor from blank samples on day 1, to almost complete separation of all species from one another and from blank samples at day 6. The fifth day measurements gave rise to a PCA scores plot in which all species, except for *P. nordicum*, were separated from the blank samples; furthermore, *G. candidum*, *P. carneum* and *P. expansum* were all grouped separately, showing differentiation of the mentioned species. *Penicillium roqueforti* could not be completely distinguished from *P. paneum* or *P. camemberti*, although slight grouping was evident. Analysis for mycotoxin production performed by LC-MS showed that 14 mycotoxins were detected from the seven species. No mycotoxins were detected in samples from *G. candidum* and *P. camemberti*.

Conclusions: Differentiation of fungi to species level has been achieved by the use of e-nose technology, thus permitting prediction of mycotoxin production by differentiating between mycotoxin-producing species and non-toxinogenic species. Furthermore, these findings support the hypothesis that volatile organic compounds can be used as biomarkers in the differentiation of fungi to species level.

Higher energy expenditure in nutritionally programmed offspring in later life in sheep

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Objective: Prepartum and early life nutrition may permanently programme offspring's subsequent metabolism in later life. Despite the fact that intermediary pathways of nutrient metabolism are increasingly known, the long-term interrelations between maternal nutrition and substrate utilization in later life at the whole-body level need much more attention. Quantitative energy metabolism and endocrinological data could enhance our understanding of the effects of early life nutrition on whole-body metabolism in later life.

Methodology: Adult twin-pregnant ewes fed either *ad libitum* (control) or 60% of their requirements (nutritional-programmed, NP, offspring) during the last trimester of gestation ($\sim$147 days). After 2 years, the offspring were fed restricted diets (60% of requirements) from day 42 prepartum until term. Around day 137 of gestation, energy expenditure (EE) and respiratory quotient (RQ) were determined from gaseous exchange (open-air-circuit respiration unit) and urinary nitrogen (UN). Data were analysed by the MIXED procedure in SAS® using a model with maternal nutrition as fixed effect, animal as random effect, and day as repeated measure.

Results and discussion: Weights of NP offspring were lower at birth compared with control offspring (4.1 ± 0.2 vs 3.4 ± 0.2 kg, $p < 0.05$).This difference persisted into adulthood, where NP offspring in late gestation weighed approx. 15 kg less than control offspring ($\sim$90 kg) ($p < 0.01$). $EE/BW^{0.75}$ (504 vs 451 kJ day^{-1})($p < 0.05$) and RQ values (0.88 vs 0.83) ($p = 0.05$) were higher in NP offspring. Glucose (2.5 vs 2.8 mmol l^{-1}) ($p < 0.05$) and β-hydroxybutyrate (0.8 vs 1.3 mmol l^{-1}) ($p < 0.01$) concentrations were, however, lower in the NP offspring and less affected by the feed restriction compared with control offspring.

Conclusions: Foetal nutritional restriction in sheep increases $EE/BW^{0.75}$, when the animal is subjected later in life to underfeeding during late gestation. Energy expenditure was to a larger extent based on carbohydrate rather than fat oxidation, despite a shift in glucose homoeostatic regulation towards a lower set-point. There was no indication of insulin resistance in the late-gestation NP offspring. Adult NP offspring appear less efficient than control offspring in utilizing body reserves (fat) when needed, such as during undernutrition in late pregnancy.

Sensory characteristics and *in vitro* evaluation of iron dialysability of a gluten-free bread fortified with iron

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Objective: The objectives were (i) to produce gluten-free bread (GFB) fortified with iron (GFB-Fe) using selected iron compounds; (ii) to test sensory characteristics (mouth-feel texture, crumb colour, odour and taste) of the GFB-Fe; and (iii) to compare the iron dialysability of various iron compounds in GFB-Fe.

Methodology: The ingredients used to make the GFB were gluten-free flour containing rice flour, maize starch, potato starch and locust bean gum, amaranth flour, egg white powder, vegetable fat powder, α-amylase, moist yeast, salt and emulsifier-DATEM. The GFB was fortified with selected iron compounds (iron pyrophosphate, iron pyrophosphate with emulsifiers, NaFeEDTA, electrolytic iron, ferrous gluconate, ferrous lactate and ferrous sulfate). Sensory analysis was performed by a trained panel (Mandala and Daouaher, Int J Food Sci Technol 2005;40:759). To compare iron dialysability of various iron compounds in GFB-Fe, all products were digested *in vitro* (Kapsokefalou and Miller, J Food Sci 1991;56:352). This *in vitro* model simulates the gastrointestinal digestion by subjecting samples to incubation for 4.5 h at 37°C, at different pH, in the presence of peptic enzymes, and by fractionating digests through the aid of a dialysis membrane. Ferrous and total iron in the dialysates were determined spectrophotometrically.

Results and discussion: The products with the most acceptable sensory properties were those fortified with ferrous pyrophosphate with emulsifiers and ferrous pyrophosphate. Ferrous dialysable iron in GFB-Fe fortified with iron pyrophosphate with emulsifiers and NaFeEDTA was higher ($p < 0.05$) than that in GFB-Fe fortified with other iron compounds. Ferrous dialysable iron (molecular weight <8000) was used as an index for prediction of iron bioavailability. Fortified gluten-free products are rare, but it has been suggested that the development of such products would improve the quality of the diet (Kupper, Gastroenterology 2005;128:121) of patients with coeliac disease.

Conclusion: This attempt at producing an iron-fortified gluten-free baked product, with satisfactory sensory and nutritional characteristics, was successful and encouraging for further studies.

Docosahexaenoic acid modulates lipid raft phospholipid composition and major histocompatibility complex class II expression in dendritic cells

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Objective: Polyunsaturated fatty acids (PUFA) have been shown to affect various aspects of the immune system. The influence of PUFA on the adaptive immune response has mainly focused on T-cells, which have been extensively investigated, whereas the influence of PUFA on dendritic cells (DCs), the main link between the innate and adaptive immunity, has been sparsely studied. The aim was to investigate the effect of docosahexaenoic acid (DHA) supplementation on modulating lipid composition in lipid rafts of DCs and on the expression of surface markers and cytokine production.

Methodology: Murine bone marrow-derived DCs were generated with and without addition of DHA during culture. Lipid rafts were isolated by discontinuous sucrose density gradient ultracentrifugation, and phospholipid fatty acid composition and cholesterol were determined. Surface marker expression on DCs was determined by flow cytometry and cytokine production was measured in supernatants from mature DCs.

Results and discussion: DHA treatment altered the fatty acid composition of lipid rafts with respect to DHA, and DHA incorporation selectively downregulated major histocompatibility complex (MHC) class II expression, whereas CD40, CD80, CD86 and cytokine production by mature DCs was unaffected by the incorporation of DHA.

Conclusions: Lipid raft membrane composition of DCs is influenced by the incorporation of physiological relevant levels of DHA into the plasma membrane, and the expression of MHC class II molecules on the surface of DCs is very sensitive towards exchanging of membrane fatty acids with DHA.

Maternal dietary long-chain n-3 polyunsaturated fatty acid during gestation and lactation reduces the specific antibody response in neonatal mice

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Objective: The effect of prenatal and postnatal maternal dietary fatty acid composition on the specific immune response in mice pups was studied.

Methodology: From the day of conception and throughout lactation dams were fed low-fat diets containing 4% fat, of which 25% was n-3 polyunsaturated fatty acids (PUFA) from linseed oil, fish oil or structured oils with the long-chain n-3 PUFA positioned in *sn*1/*sn*3 or in *sn*2. A control group receiving a saturated diet with no n-3 PUFA was included. Pups were injected with ovalbumin within 24 h of birth. Three weeks later blood and spleens were collected for determination of plasma antigen-specific antibody, spleen cell proliferation and cytokine production *ex vivo* in the pups, as well as fatty acid analysis of spleen cell phospholipids and surface marker expression on spleen cells for both pups and dams.

Results and discussion: The fatty acid composition of pup spleens reflected the maternal diet, with higher levels of n-3 PUFA in all experimental groups compared with the control group. Incorporation of 20:5n-3 and 22:6n-3 in spleen cell

phospholipids was not influenced by the position in the dietary triacylglycerol. Maternal dietary long-chain n-3 PUFA gave a reduced antigen-specific antibody response in the pups compared with pups of dams fed both saturated fatty acids and α-linolenic acid.

Conclusions: Maternal dietary long-chain n-3 PUFA influence the immune response in pups, independent of the position in the dietary triacylglycerol.

Early determinants of body composition in 17-year-old adolescents from a Danish cohort of healthy term infants

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Objective: The aim was to examine the effect of size and weight gain in infancy in healthy term infants on body composition in adolescence, measured as body mass index (BMI), body fat percentage and waist circumference.

Methodology: The Copenhagen Cohort Study on Infant Nutrition and Growth, a prospective observational study, investigated 143 healthy term infants. Only infants with normal birth weight (10th to 90th centiles) were included. Weight at birth and at 9 months were used in this analysis. At 17 years a follow-up was done including anthropometric measurements, and whole-body dual-energy X-ray absorptiometry scan (Hologic 1000/W). Complete data existed on 105 subjects. Linear regression was used to examine the association between birth weight, weight gain from 0 to 9 months, weight at 9 months and parameters of body composition at the age of 17 years.

Results and discussion: 8.6% (four males, five females) and 1.9% (one male, one female) of the participants were overweight or obese, respectively, at 17 years of age. There was no significant association between birth weight and any body composition parameters. Both weight at 9 months and weight gain from birth to 9 months were positively associated with BMI at 17 years. Weight gain from birth to 9 months was borderline-significantly associated with body fat percentage at 17 years. These effects were independent of birth weight. Waist circumference at 17 years, controlled for current BMI, was related to neither weight at 9 months nor weight gain from birth to 9 months. The findings suggest that birth weight is not a predictor of overweight and body fat, in a population of children with normal birth weight. However, infancy weight gain seems to be important. There is a need for more data on the long-term effects of infant growth (distinguishing between weight, length and body composition) and on how diet during infancy influences these aspects of infant growth, before any changes in recommendations on infant diet can be made.

Conclusions: In term infants with normal birth weight followed up at the age of 17 years, birth weight is not associated with BMI or body fat percentage, while weight gain from 0 to 9 months is positively associated with BMI ($p < 0.001$) and body fat percentage ($p = 0.078$).

The effect of seven day supplementation with milk protein fractions and milk-minerals on insulin-like growth factors and glucose–insulin metabolism in Danish prepubertal boys

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Objective: A previous study found a positive association between milk consumption, insulin-like growth factor-1 (IGF-1) and height in 2.5-year-old children, and that increase in milk intake in 8-year-old boys increased the growth factors IGF-1 and insulin, which was not the case with an increased meat intake, with the same amount of animal protein. Thus, milk but not meat seems to contain some yet unknown growth-promoting constituents. The objective of this study is to examine the effect of increased intake of casein and whey protein with and without milk-minerals on the growth factors IGF-1 and insulin.

Methodology: This study had a double-blinded, randomized, 2×2 factorial design in which 60 8-year-old boys were randomized to receive one of four milk-based drinks daily for 7 days: whey; whey plus extra milk-minerals (calcium and phosphate); casein; and casein plus extra milk-minerals. In addition, they were asked to eat their normal diet *ad libitum*. The children were examined at baseline and after intervention. Fasting serum IGF-1, IGF binding protein-3 and insulin were determined with Immulite 1000 and glucose with Cobas. Insulin resistance was calculated with the homoeostasis model assessment.

Results and discussion: IGF-1 increased significantly in the casein group ($p < 0.0001$) and non-significantly in the whey group. The increase in IGF-1 in the whey group was larger than the increase in the casein group ($p = 0.007$). IGF-1 increased significantly in the low milk-mineral group ($p = 0.005$) and in the high milk-mineral group ($p = 0.01$), with no difference between the milk-mineral groups ($p = 0.78$). Insulin ($p = 0.006$) and insulin resistance ($p = 0.01$) increased significantly in the whey group and non-significantly in the casein group, with no difference between the milk protein groups ($p \leq 0.50$). Insulin ($p = 0.02$) and insulin resistance ($p = 0.03$) increased significantly in the high milk-mineral group and non-significantly in the low milk-mineral group, with no difference between the milk-mineral groups ($p = 0.09$ and $p = 0.14$, respectively).

Conclusions: This study demonstrates that casein stimulates IGF-1 and whey stimulates insulin, with no additional effects of milk-mineral.

Investigating the effect of n-3 dietary fatty acids on liver phospholipid composition by liquid chromatography–mass spectrometry

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Objective: Many studies have shown that n-3 fatty acids have beneficial effects for human health such as decreasing the production of inflammatory cytokines and limiting diet-induced obesity. However, the mechanisms are not fully understood. Since many biological processes occur at the plasma membrane and the functionality of membrane lipids is determined by their local concentration, the present study aims to investigate how dietary n-3 fatty acids affect membrane phospholipid composition.

Methodology: Male albino Wistar rats were fed with a diet containing 10 wt% fat (butter, linseed oil or fish oil) for 3 weeks. The liver lipids were extracted by chloroform and methanol. The total lipids were analysed by liquid chromatography–mass spectrometry (LC-MS) using electrospray in the negative mode, phospholipids were separated on a silica column and their molecular species were identified by molecular or pseudomolecular ions and fatty acid fragment ions.

Results and discussion: Dietary lipids affected the molecular species of all major phospholipids, i.e. phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI) and sphingomyelin (SM). For the butter group 18:0/20:4 was the most abundant phospholipid species, representing 40.3% of total PE, 24.9% of PC, 66.7% of PS and 54.6% of PI. The linseed oil group had lower level of 18:0/20:4 (34.8% of PE, 20.5% of PC and 5.7% of PS), whereas 18:0/22:6 increased to 13.2% for PE, 6.8% for PC, 26.9% for PS and 7.4% for PI. The level of 18:0/22:6 increased more significantly after the fish oil diet, especially for PS and PI. It represented 42.6% of PS and 13.6% PI. 16:0/20:5 was only detected in the fish oil group and it constituted 6.3% of PE and 9.1% of PC. A low level of lyso PC was also detected; it only contained 16:0 and 18:0. Translocation of a protein to the plasma membrane in response to the generation of polyphosphoinositol lipids is believed to be an important component of cellular regulation, therefore the significant increase in PI 18:0/22:6 may play an important role in this context.

Conclusions: LC-MS is a good tool for the identification and quantification of membrane phospholipid species. Dietary n-3 fatty acids result in changes of PL molecular species in liver, and n-3 fatty acids are incorporated into PS and PI to a much higher degree than the other phospholipids.

Control and freedom: going shopping with weight losers

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Objective: In order to understand how dietary guidelines work and how they affect the recipients, it is not enough to explore the biochemical effects of different nutritional compositions. From a social scientific perspective it is of pivotal importance not only to explore how the messages are meaningfully interpreted by the recipients, but also to examine the situations in which people are directly confronted with food and choices regarding what and what not to buy, prepare and eat.

Methodology: Data are presented from an ethnographic study of food shopping among people who want to change their eating practices to lose weight or maintain a weight loss. The data were constructed through 12 sessions of participatory observation among five young adults who participated in a dietary intervention trial (the MUFObes project) carried through at the Department of Human Nutrition, Royal Veterinary and Agricultural University, Denmark.

Results and discussion: The findings are discussed in relation to results from other studies of consumption, identity formation and weight-loss strategies. In comparison to most social scientific and psychological studies of weight loss, which focus on striving for self-control as the crucial goal in the identity formation of people who want to lose weight, it is argued that the focus on consumption practices opens our eyes to the importance of the concept of freedom.

Conclusions: Interpretations of the data are preliminary, as they are the outcome of an ongoing PhD study. Therefore, the presentation contains no final conclusion. However, it is argued that people who identify as and are identified as overweight differ from "normal-weight" people in the way in which they balance freedom and control in their food consumption practices.

Children's influence on family decision making in food buying and consumption with a focus on healthy food products

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Objective: The aim of this research project was to investigate children's participation in and influence on various decision stages and areas in family food buying and consumption, with a focus on healthy food products such as fruit and vegetables.

Methodology: This mixed methods study combined empirical methods based on ethnographic participant observation with quantitative questionnaires designed for Danish children aged 10–12 years and for their parents.

Results and discussion: Both parents and children participate actively in family food decision making. Children have quite a strong influence on family food buying in general and even more on specific decision areas such as those of relevance to the child.

Conclusions: From a marketing perspective, companies may benefit from knowledge about children's influence on family food decision making, regarding barriers and motives for choosing healthy food. This may also help to prevent overweight and lifestyle-related diseases.

Health effects of foods enriched with β-glucans from cereals

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Objective: Cereal β-glucans found in oats and barley has been shown to have positive health effects on lipid and glucose metabolism. Thus, people at risk of developing cardiovascular diseases or diabetes may benefit from a variety of palatable food products containing β-glucans being available. In an EU project (QLK1-2000-00535), different β-glucan-enriched foods have been developed and the health effects studied.

Results and discussion: In a study by Biörklund et al. (2005) a daily intake of a beverage containing 5 g of β-glucans from oats significantly lowered the total serum cholesterol concentration and the postprandial glucose and insulin response compared with a control beverage in subjects with increased cholesterol levels. No significant serum lipid or postprandial changes compared with control were obtained after the consumption of either 5 or 10 g of barley β-glucans or 10 g oat β-glucans. Soups enriched with β-glucans from oats did not show any significant health effects in comparison to control soups in subjects with increased cholesterol levels or in diabetic subjects (Biörklund, submitted). The liquid products used in the studies were all enriched with low molecular weight β-glucans, increasing the amounts that could be incorporated per serving.

Conclusion: It could be of further interest to study the optimal mode of consuming β-glucans.

Bioavailability and metabolism of hesperetin and naringenin from enzymically or non-enzymically treated orange juice

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Objective: Citrus flavonoids such as hesperetin and naringenin have been shown to protect against lifestyle-related diseases. When these compounds are absorbed they undergo conjugation, such as glucuronidation and sulphonation. Their health-promoting effect is therefore most likely to be due to their metabolites. Consequently, it is of great importance to investigate their bioavailability and metabolism.

Methodology: Sixteen volunteers completed a double-blind, randomized, cross-over study (Nielsen et al., in press). Subjects randomly consumed 5 ml kg^{-1} body weight of each of the following three preparations of orange juice: (i) orange juice with natural content of hesperetin- and naringenin-rutinosides; (ii) enzymically treated orange juice to yield equivalent amounts of hesperetin- and naringenin-7-glucoside; and (iii) orange juice fortified with hesperetin-7-rutinoside to obtain a three-fold increase in the concentration of this rutinoside than naturally present in the juice.

Results and discussion: Consumption of the enzymically treated orange juice containing the glucoside compared with the natural orange juice containing the rutinoside resulted in higher absorption of naringenin, as it did for hesperetin (Nielsen et al., in press). In urine the metabolic profile was found to be slightly different among the three treatments. This indicates that different metabolic pathways are involved when the sugar moiety of the flavonoid is altered, resulting in changes in the absorption site from the ileum to the colon. An increase in the dose of the rutinosides was found to affect the metabolite profile in urine.

Conclusions: The bioavailability of both hesperetin and naringenin is increased by conversion of their respective rutinosides to glucosides, and the resulting absorption site affects the profile of the metabolites formed and excreted in urine. This has a significant impact for future interventions on the health benefits of citrus flavonoids.

Data mining on the relationship between health-related responses and taste of bioactive peptides derived from food proteins

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Objective: The aim was to explore possible relationships between health-related responses and the taste of bioactive peptides.

Methodology: Data mining was conducted on health-related responses, such as angiotensin I-converting enzyme (ACE) and prolyl oligopeptidase (POP) inhibition, and taste responses, such as bitter and unami, of peptides derived from food proteins. Available peptide quantitative structure–activity relationship (QSAR) models were used to estimate possible relationships between these important, but physiologically very different, human responses in relationship to bioactive peptides.

Results and discussion: Bioactive peptides derived from proteolysis of food proteins have shown to have physiological effects beyond being a source of energy and essential amino acids. Possible health-related responses on humans have been related to ACE and POP inhibition, mineral binding, opioid and antimicrobial effects. Future foods for health could therefore contain specific

peptide fractions from hydrolysed food proteins as functional food ingredients aimed at individuals with certain deficiencies or lifestyle-related diseases. However, peptides from hydrolysed food proteins can also have taste properties. For instance, a bitter taste may be a real problem when using casein hydrolysates in foods. If peptides with health-related effects are to be used in novel functional foods, it is important to explore their influence on sensory properties. There are already extensive experimental data in the research literature on bioactive health-related effects or on the taste of peptide structures. However, to the authors' knowledge the health-related effects and taste of peptides have not been studied simultaneously and compared. As an initial approach to this field a data-mining study using available peptide QSAR models to estimate possible relationships between health effects and the taste of bioactive peptides will be presented.

Conclusions: Estimation of taste for ACE and POP inhibitory peptides using available peptide QSAR models, and equivalently estimating ACE and POP inhibition of bitter and unami peptide structures, shows that taste aspects will be important if such bioactive peptides are to be used as functional ingredients in future foods for health.

Conjugated linoleic acid and vaccenic acid have no effect on blood pressure and isobar arterial elasticity in healthy young men

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Objective: The aim was to examine the effect on blood pressure and isobar arterial elasticity (AE_I) as a measure of arterial health of a commercial mixture of Conjugated linoleic acid (CLA) and of milk fat produced to have a high content of vaccenic acid (VA).

Methodology: Sixty healthy young men participated in this double-blinded, randomized, 5 week parallel intervention study. The participants substituted 115 g of their daily fat intake with fat from one of three test diets: $D_{CLA\text{-}mix}$, rich in CLA (4.7 g day^{-1} of *c*9,*t*11- and *t*10,*c*12-CLA isomers in equal amounts); $D_{Vaccenic}$, rich in VA (4.1 g day^{-1}); or $D_{Control}$, a control diet with a low content of VA and CLA. Blood pressure and AE_I (measured by an oscillometric method) was measured before and after the intervention period.

Results and discussion: There was a tendency for a higher systolic blood pressure after $D_{CLA\text{-}mix}$ compared with $D_{Control}$ ($p=0.07$), but no difference in the effect of the test diets on diastolic blood pressure ($p=0.37$) or pulse pressure ($p=0.80$). There was no difference in isobar arterial compliance ($p=0.52$), distensibility ($p=0.71$) or volume ($p=0.52$).

Conclusions: In conclusion, diets rich in milk fat and either CLA or VA had no adverse effect on blood pressure or AE_I in healthy young men compared with a control diet.

Interaction between alcohol intake and oxidative defence in breast cancer

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Objective: Breast cancer may be related to oxidative stress. Lower antioxidant enzyme activity has been reported in breast cancer patients compared with healthy controls, and the polymorphism *GPX1* Pro198Leu has been associated with the risk of lung and breast cancer. The aims of this nested case–control study were to determine whether *GPX1* Pro198Leu and glutathione peroxidase (GPX) activity in prospectively collected blood samples are associated with breast cancer risk among postmenopausal women and whether GPX activity levels are associated with other known breast cancer risk factors.

Methodology: Within the prospective Diet, Cancer and Health study of 57 000 Danes, 377 female breast cancer cases were matched with 377 controls.

Results and discussion: Carriers of the variant *T*-allele of *GPX1* Pro198Leu were at 1.43-fold higher risk of breast cancer than non-carriers (95% confidence interval 1.07–1.92). Prediagnostic GPX activity tended to be lower in cases than in controls. GPX activity was positively correlated with intake of alcohol ($p<0.0001$) and the catalytic activity was lowered by 5% for each additional copy of the variant *T*-allele ($p=0.0003$). Alcohol intake was correlated with increased GPX activity for the *C*-allele but not for the *T*-allele.

Conclusions: Results from this prospective study suggest that the *GPX1* Pro198Leu-associated lowered GPX activity is associated with higher breast cancer risk among Danish women. The risk is differentially affected by alcohol in the carriers and non-carriers of the variant allele.

Effect of long-term selenium yeast supplementation on biomarkers of oxidative defence in healthy elderly volunteers

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Objective: Selenium (Se) supplementation has been reported to reduce the incidence of cancer. The protective mechanisms are not known. They may be related to beneficial actions of selenoproteins or to induction of phase II metabolizing genes involved in detoxification. The aim of this study was to determine the effect of Se supplementation on the induction of protective enzymes.

Methodology: Five-hundred participants aged 60–74 years were randomized to three dosage levels, 100, 200 or 300 μg of Se as selenized yeast (SelenoPrecise; PharmaNord, Denmark) per day or placebo. After 5 years of supplementation, expression of *GPX1*, *GCLC*, *Fra1*, *AhRR* and *NQO1* in lymphocytes and activity of GPX, GR and GST in plasma, red blood cells or thrombocytes were determined in a random subsample of 107 participants.

Results and discussion: Plasma Se concentration increased linearly with increasing Se intake ($p < 0.0001$). No effect was found of Se supplementation on activities of GPX, GR or GST in red blood cells, plasma or thrombocytes. With a mean plasma Se concentration in the placebo group of 9.2 μg dl^{-1}, one would expect saturation of plasma and red blood cell GPX activity. When the Se-supplemented groups were pooled and tested against the placebo group, there was significantly higher thrombocyte GPX activity in the Se-supplemented group compared with controls. Thrombocyte GPX activity is known to saturate at higher plasma Se concentrations compared with red blood cell or plasma GPX. No effect of Se supplementation was found on gene expression in lymphocytes.

Conclusions: Plasma Se concentrations were not associated with the investigated biomarkers. Selenium supplementation as selenized yeast did not influence enzyme activities of GPX, GR or GST in red blood cells, plasma and thrombocytes, or gene expression of *GPX1*, *GCLC*, *Fra1*, *AhRR* or *NQO1* in lymphocytes in subjects with an adequate Se intake.

Casein-stimulated bone growth and whey-stimulated tissue growth in newborn immunocompromised pigs

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Objective: Milk nutrients stimulate growth of newborn infants, but little is known about the mechanisms or the role of specific milk proteins (casein, whey proteins) relative to plant proteins (e.g. soya products). Even less is known about the effects of milk nutrients in the diseased newborn infant, which also has a very nutrient-sensitive intestine. This question was investigated in a pig model of immunocompromised infants. Colostrum-deprived piglets are known to suffer from slow growth and immune disorders in the immediate postnatal period owing to a lack of passive immunization and growth-promoting nutrients in sow's colostrum.

Methodology: Term pigs were delivered naturally following induction of labour. They were fitted with oesophageal feeding tubes and fed milk diets (15 ml kg^{-1} 1.5 h^{-1}) containing different protein sources (casein, CASE, $n = 6$; whey, WHEY, $n = 12$; soya protein hydrolysate, SOY, $n = 5$). The diets were mixed to mimic the nutritional composition of sow's milk and had the following macronutrient contents (g 100 ml^{-1}): 7.6 fat, 5.5 protein, 5.3 lactose, 0.19 Ca, 0.14 P. Body composition and whole-body bone mineral content in each pig were assessed under anaesthesia using dual-energy X-ray absorptiometry (DEXA scanning) at the start and end of the 7 day experiment. At tissue collection, internal organs were excised, measured and weighed. Furthermore, to assess intestinal maturation, brush-border enzyme activities were measured in the proximal, middle and distal small intestine.

Results and discussion: WHEY pigs gained $21 \pm 3\%$ of initial body weight, CASE pigs $10 \pm 3\%$ ($p < 0.05$) and SOY pigs $-2.3 \pm 6.3\%$ ($p < 0.01$ vs WHEY). Dual-energy X-ray absorptiometry scanning showed a higher whole-body tissue increment for WHEY pigs (347 ± 43 g vs CASE 208 ± 47 g and SOY 63 ± 47 g, $p < 0.05$), resulting from increases in both lean mass and fat. CASE pigs had a higher increase in whole-body bone mineral content (5.4 ± 1.2 g) relative to WHEY (2.3 ± 1.1 g, $p < 0.05$), resulting from increased bone surface and bone mineral densities. While the CASE and SOY pigs had heavier stomachs compared with WHEY pigs (10.5 ± 0.7 vs 6.4 ± 0.5 g kg^{-1}, $p < 0.001$), the relative weight of the small intestine was higher in CASE (38.5 ± 1.7 g kg^{-1}) and WHEY (37.9 ± 1.2 g kg^{-1}) pigs compared with SOY pigs (32.9 ± 1.5 g kg^{-1}, $p < 0.05$). Further, the SOY pigs had decreased mucosal mass ($p < 0.05$), despite no significant treatment differences in villus height (709 ± 49 μm), villus width (91 ± 3 μm) or crypt depth (181 ± 5 μm). Casein stimulated aminopeptidase A activity across the entire intestine and also dipeptidylpeptidase IV in the proximal part ($p < 0.05$ relative to WHEY and SOY). SOY pigs had elevated sucrase and maltase activities ($p < 0.05$).

Conclusions: Relative to soya protein, whey and casein proteins stimulated small intestinal growth and function in newborn immunocompromised pigs. However, casein increased bone mineralization and intestinal function more than whey, while whey proteins stimulated soft-tissue growth (organs, muscle and fat). The results indicate how the source of protein affects body and organ growth responses in immunocompromised neonates. The results are important to design optimal diets for sick newborn infants.

Amniotic fluid decreases the incidence of necrotizing enterocolitis in preterm pigs

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Objective: Preterm infants are susceptible to gastrointestinal disorders such as necrotizing enterocolitis (NEC). Risk factors for NEC include prematurity, formula feeding and bacterial colonization. Together these factors result in an exaggerated inflammatory response, leading to haemorrhagic inflammatory necrosis of the gastrointestinal tract. Amniotic fluid (AF), the foetal "diet", contains immunomodulatory components, but it remains unknown whether AF has beneficial effects after birth. The aim was to study the progression of formula-induced NEC in a preterm pig model and the effects of enteral AF administration.

Methodology: Thirty-six preterm (93% gestation) pigs were delivered via Caesarean section and maintained on total parental nutrition (TPN) for the first 48 h, then fed either sow's colostrum (COLOS) or formula (FORM). Pigs were killed at 0, 8 and 17 h after enteral feeding and the gastrointestinal tract was evaluated for signs of NEC. In a follow-up study, 30 preterm pigs were allocated to three groups: sow's colostrum (COLOS), formula (FORM) or formula plus AF (F-AF). F-AF pigs were orally administered pure AF throughout the TPN period and formula plus AF during the enteral feeding period (12–40 h). At tissue collection the gastrointestinal tract was evaluated for NEC-like lesions. A pathological score ≥ 2 (range 0–4) in any region of the gastrointestinal tract was defined as NEC.

Results and discussion: At 8 and 17 h after enteral feeding, 5/8 and 8/10 FORM pigs suffered from NEC, respectively. In the follow-up study, 7/13 FORM pigs suffered from NEC, whereas only 1/10 F-AF pig suffered from NEC. All COLOS pigs remained healthy. Many bioactive factors, such as anti-inflammatory cytokines, growth factors and antimicrobial peptides, present in colostrum are also known to be present in AF, and protect and stimulate growth in the foetal gastrointestinal tract. These observations indicate that AF is also protective against formula-induced inflammatory gastrointestinal tract disorders in preterm neonates.

Conclusions: Preterm formula-fed pigs are very sensitive to gastrointestinal tract lesions, with the onset of NEC occurring as early as 8 h after enteral feeding. Oral administration of AF appears to have protective properties, not only in foetal, but also in neonatal pigs, as indicated by a decreased incidence of NEC in F-AF pigs.

Mode of delivery affects diet-induced small intestine atrophy and dysfunction in preterm pigs

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Objective: In preterm neonates, total parenteral nutrition (TPN) followed by formula feeding can cause small intestinal mucosal atrophy and dysfunction, potentially leading to necrotizing enterocolitis (NEC). It is unknown whether this susceptibility is affected by mode of delivery at birth. In contrast to caesarean delivery, vaginally delivered neonates are exposed to elevated levels of glucocorticoids, putative stimulators of small intestine development, and a more stable maternally influenced microbiota. A preterm pig model was used to determine whether vaginal delivery could prevent TPN and formula-induced small intestinal atrophy and dysfunction.

Methodology: Preterm pigs (93% gestation) were delivered vaginally (VD) or via caesarean section (CS), maintained on TPN for 36 h, then fed sow's colostrum (COL) or infant milk formula (FORM) for 48 h.

Results and discussion: NEC lesions were increased in FORM groups and minimal in COL groups. Relative to VD-FORM, distal small intestinal villus heights increased by 14% in CS-FORM and VD-COL and increased by 43% ($p < 0.05$) in CS-COL. Relative dry weights of the small intestinal mucosal layer were lower (10–14%, $p < 0.01$) in VD-FORM pigs than in the other treatment groups. Compared with VD-FORM, aminopeptidases A and N activities were increased in VD-COL and CS-FORM (20–25%) and increased in CS-COL (37%, $p < 0.05$). Lactase and maltase activities were lowest in VD-FORM, higher in CS-FORM (22–27%) and highest in both COL groups (45–50%, $p < 0.01$). TPN- and formula-induced small intestinal atrophy and dysfunction were most pronounced in VD pigs, compared with CS pigs.

Conclusions: The premature small intestine may be more susceptible and respond inappropriately to the endocrine changes and microbial colonization patterns of vaginal delivery.

Perceived importance of healthiness in meals

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Objective: The study aims to investigate consumers' food choice in order to understand how the perception of healthiness of meals influences decision making. The findings will be instrumental in developing healthier alternatives that can serve as acceptable substitutes for preferred food products. Food choice is context dependent. Two important factors influencing food choice differently depending on context are health and pleasure. To balance the diet, consumers apply strategies that uphold the impression of a balanced and healthy diet, but still allow for indulgence.

Methodology: The study applied two group processes to elicit information about food choice and perceived healthiness. Three groups of food experts ($n = 15$) developed a set of food items to represent healthy and less healthy dinner options for two contexts, an everyday meal and a weekend meal with friends. In two sessions, six groups of three people first constructed a meal that they would like to serve in one of the two situations, and then modified the meal according to their perceptions of increasing the healthiness of the meal.

Results and discussion: In the constructed weekend meals approx. 60% of the items were considered unhealthy. In the proposed everyday meals 50–80% of the items were considered unhealthy. During the discussion the consumers all

agreed that they allowed more unhealthy food items for the weekend meals, but compensated with eating healthier during the week. The meals that the respondents constructed did not confirm this view. The main differences in food items between the contexts were that more processed foods were chosen for the everyday meal, and the subjects considered processed or ready-made foods to be more unhealthy than home-made or less processed foods. The consumers' perception of healthiness was centred on the contribution of healthy or unhealthy nutrients in single products and not seen in the context of a full meal. The health-modified menus were not viewed favourably, and the consumers did not think that they would serve these options.

Conclusions: There are opportunities for the development of healthier versions of processed food products that are customarily chosen for everyday meals. Costs of the items will be a limiting factor for purchase. A major challenge for the food industry is the perception than commercially produced food is viewed with suspicion.

Tomato consumption in Lithuania

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Objective: The aim was to compare the consumption of tomatoes in Lithuania and other European countries.

Methodology: The evaluation of tomato consumption was based on the data of tomatoes grown, exported and imported. Tomatoes and tomato-based foods (juice, sauce, paste, ketchup, canned tomatoes) are useful for their chemical composition. They are excellent sources of potassium, folic acid, and vitamins A, C and E. Tomatoes are also rich in other phytocompounds, including carotenoids and polyphenols. Predominant carotenoids in tomatoes are lycopene (a red pigment) and betacarotene (provitamin A). Phytofluene and phytoene, the precursors of lycopene, are also found in tomatoes. Lycopene, the predominant carotenoid in tomatoes, is the major carotenoid in human serum, although it as not synthesized by the human body; it must be consumed with food. Recent interest in lycopene and tomatoes and their potential disease-preventing properties began when Giovannucci et al. (1995) demonstrated an inverse relationship between dietary intake of carotenoids and prostate cancer incidence. A couple of years later lycopene concentrations in adipose were inversely associated with risk for myocardial infarction.

Results and discussion: The average intake of tomatoes and tomato products ranges from 15 to 150 g day^{-1} in European countries. This study evaluated the consumption of tomatoes in Lithuania according to data on their growth, export and import. These data showed that tomato consumption in Lithuania is the lowest among the European countries.

Conclusion: For dietary recommendations, the results suggest that consumption of tomatoes in Lithuania must be at least doubled.

Myotubes as a model for studying the effects of creatine, analysis of metabolites and protein regulation

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Objective: The aims were to examine underlying mechanisms for increased weight gain following dietary creatine monohydrate (CMH) supplementation, using differentiated muscle cell cultures (myotubes) as a model.

Methodology: Differentiated porcine satellite cell cultures were used as a model for examining the effects of CMH on protein synthesis and degradation by the incorporation/release of [^{3}H]tyrosine. In addition, the effect of CMH supplementation on protein regulation and metabolism was investigated in differentiated C2C12 muscle cell cultures by (i) two-dimensional gel-based proteomics and (ii) high-resolution ^{1}H-MAS nuclear magnetic resonance (NMR) metabolic profiling. Values of relative spot volumes and NMR peaks were analysed by multivariate statistics including principal component analysis (PCA), discriminant partial least squares (D-PLS) and PLS2.

Results and discussion: It was shown earlier that dietary supplementation of CMH increased muscle growth in pigs. This study on primary porcine differentiated muscle myotubes demonstrated that addition of CMH increases muscle growth by increasing the rate of protein synthesis, while having no effect on protein degradation. Moreover, this study is the first to report the combined use of proteomics and high-resolution ^{1}H-NMR for the characterization of cell cultures. The variation in protein expression was larger in the control cells than in the CMH-treated cells as analysed by PCA. By D-PLS six protein spots were found to be significantly different between CMH-treated cells and control cells. D-PLS on the obtained NMR spectra revealed that control and CMH-treated cells could be discriminated by differences in their metabolic profile. Correlation analysis (PLS2) between NMR spectra and the six proteomic spots identified correlations between specific NMR regions and these protein spots, implying an association between metabolic profile and protein regulation.

Conclusions: *In vitro* investigations into the effects of CMH on differentiated myotubes indicated that the increased muscle growth observed *in vivo* was due to increased protein synthesis. Furthermore, it was possible to differentiate between controls and myotubes exposed to CMH by both metabolic and proteomic analysis.

Human colon cells show biphasic survival characteristics in response to increasing falcarinol concentrations

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Objective: The aim was to investigate *in vitro* the possible mechanisms involved in the protective effect of falcarinol on the development of azoxymethane-induced colon preneoplastic lesions, recently shown in rats.

Methodology: The polyacetylene falcarinol was isolated from ethyl acetate extracts of carrot roots by column chromatography and preparative high-performance liquid chromatography. Human colon adenocarcinoma cells (Caco-2) were used as a model for investigating the effects of falcarinol on (i) cell proliferation by cell counts and incorporation of [^{3}H]thymidine; (ii) apoptosis by antibody-mediated flow cytometric detection of the active form of caspase-3 and determination of altered cell size, internal complexity and the extent of cell detachment; and (iii) DNA damage by single-cell gel electrophoresis (SCGE).

Results and discussion: One of the mechanisms that may be involved in the protective effect of falcarinol against the development of azoxymethane-induced colon preneoplastic lesions in the rat model is the ability of cells to undergo apoptosis. At relatively low falcarinol concentrations, the expression of the apoptosis indicator caspase-3 was decreased (1–10 μM) concomitantly with decreased DNA strand breaks (0.5 μM). Cell proliferation was increased (1–10 μM) and cellular attachment was unaffected ($<$10 μM). These results may indicate a slight prosurvival effect on Caco-2 cells at low concentrations of falcarinol. At concentrations above 20 μM the proliferation of Caco-2 cells was decreased, and the number of cells expressing active caspase 3 increased, simultaneously with increased cell detachment. DNA damage was increased significantly above 10 μM falcarinol exposure, together with a high degree of fragmentation, and condensation of the remaining non-fragmented DNA was observed. Thus, when exposed to falcarinol at concentrations above 20 μM, Caco-2 cells seemed to exhibit apoptotic characteristics.

Conclusions: The effects of falcarinol on Caco-2 cells seemed to be biphasic, exhibiting proliferative and apoptotic characteristics, at low and high concentration of falcarinol, respectively. The potential for the manifestation of such divergent effects must be considered before approaching the development of dietary supplements.

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