

Nutrigenomics – new approaches for nutrition, food and health research

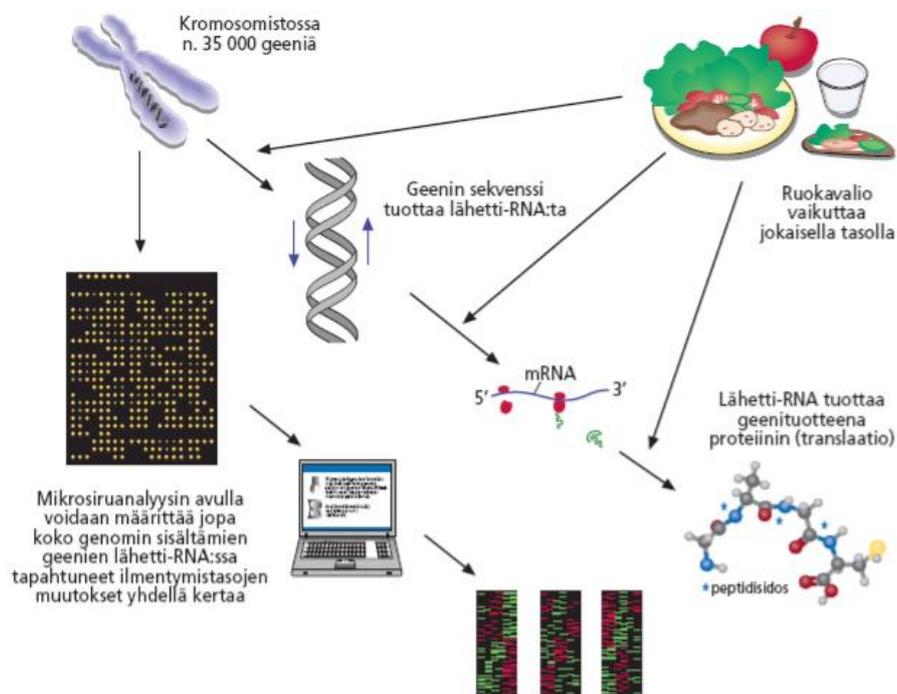
Nutrigenomiikka - merkitys ravitsemus- ja elintarviketutkimuksessa

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Preface

Nutrigenomics, the use of systems biology approach in nutrition, has attracted both scientists and industrialists increasingly during this millennium. University of Kuopio is focused on health and well-being, and has a strong background in clinical nutrition. Gene-nutrient interactions have already long been a key research topic. Several projects have also been started using the nutrigenomics approach, and the Kuopio Nutrigenomics Centre was established in 2005.

This report was produced by Food and Health Research Centre ETTK by commission of Technology Centre Teknia Ltd/Kuopio Centre of Expertise in Wellbeing. The report is expected to assist in developing the future nutrigenomics activities in the Kuopio Science Park, and also to catalyze discussions about the opportunities in personalized nutrition. We thank Dr. Anneli Tuomainen for initiating the work, and all our colleagues in Kuopio for their input.

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The authors

1 Introduction

While traditional nutrition research concentrated on nutrient deficiencies and impairment of health, it nowadays focuses on improving health through diet. Over the past few decades, epidemiological, clinical and mechanistic studies have indicated many relations between nutrition and health. Links have been established between dietary habits and degenerative diseases like cardiovascular diseases, type 2 diabetes and cancer. Unbalanced nutrient intakes are known to be associated with the development of chronic diseases demonstrating that dietary chemicals have direct effects on molecular genetic processes.

Throughout our lifetime we are exposed to a complex mixture of foods with thousands of different compounds, and that makes diet the most important environmental factor challenging our biological system. The recognition that nutrients and other food components have the ability to interact and modulate molecular mechanisms underlying an organism's physiological functions has prompted a revolution in the field of nutrition. The creation of nutrigenomics and nutrigenetics, two fields with distinct approaches to elucidate the interaction between diet and genes but with a common ultimate goal to optimise health through the personalization of diet, provides powerful approaches to unravel the complex relationships between nutritional molecules, genetic polymorphism, and the biological system as a whole.

As a new science, nutrigenomics brings along new terminology, novel experimental techniques and a fundamentally new approach to nutrition research, such as high-throughput technologies that enable the global study of gene expression in a cell or organism. The advance of nutrigenomics has created enormous opportunities to deepen our understanding of how nutrients modulate gene expression, protein biosynthesis and metabolism.

This report first gives a short introduction to nutrient-gene interactions and the novel technologies employed in nutrigenomics research. During the past few years, numerous general papers or review articles have been published in the field of nutrigenomics (some of them are listed in Literature section), and this report is mainly based on them. The report also describes the academic centres, consortia and networks in the field of nutrigenomics as well as nutrigenomics-related activities in EU programmes and recent developments in nutrigenomics research, based on Internet searches. The current research interests at the Kuopio campus are shortly described. Finally, expected impacts of nutrigenomics on nutrition science, education, dietary counselling, commercial applications and business opportunities are discussed.

2 Nutrient-gene interactions

Genes are turned on and off according to metabolic signals that the nucleus receives from internal factors, e.g. hormones, and external factors, e.g. nutrients, which are among the most influential of environmental stimuli (Harland 2005). Early in evolutionary development, the nutrients that organisms ingested functioned as primitive signals that turned on and off pathways of synthesis or storage during periods of starvation or excess. As simple organisms developed into more complex forms of life, they retained the ability to respond to nutrient or nutrient/hormonal signals that govern the expression of genes encoding the proteins of energy metabolism, cell differentiation and cell growth. Genomes evolve in response to many types of environmental stimuli, including nutrition. Therefore, the expression of genetic information can be highly dependent on, and regulated by, nutrients, micronutrients, and phytochemicals found in food (Kaput et al 2005). Unbalanced diets alter nutrient-gene interactions, thereby increasing the risk of developing chronic diseases. The

shifting balance between health and disease states involves the complex interplay of genes and the environment, which includes diet.

Numerous dietary components can alter genetic events, and thereby influence health. In addition to the essential nutrients, such as carbohydrates, amino acids, fatty acids, calcium, zinc, selenium, folate, and vitamin A, C and E, there is a variety of nonessential bioactive components that seem to significantly influence health (Corthésy-Theulaz et al 2005, Trujillo et al 2006). These essential and nonessential bioactive food components are known to modify a number of cellular processes associated with health and disease prevention, including carcinogen metabolism, hormonal balance, cell signalling, cell cycle control, apoptosis, and angiogenesis. Often bioactive food components will modify several processes simultaneously.

Dietary chemicals can affect gene expression directly or indirectly (Kaput et al 2004, Kaput & Rodriguez 2004). At the cellular level, nutrients may: 1) act directly as ligands for transcription factor receptors; 2) be metabolised by primary or secondary metabolic pathways, thereby altering concentrations of substrates or intermediates involved in gene regulation or cell signaling; or 3) alter signal transduction pathways and signaling.

From the molecular standpoint, nutrients are considered to be signalling molecules that, through appropriate cellular sensing mechanisms, result in translation of these dietary signals into changes in gene, protein, and metabolite expression (Müller & Kersten 2003, Afman & Müller 2006). Minor changes in structure (saturated vs. unsaturated fatty acids, cholesterol vs. plant sterols) can have a profound influence on which sensor pathways are activated.

Transcription factors are the main agents through which nutrients influence gene expression (Müller & Kersten 2003, Corthésy-Theulaz et al 2005, Afman & Müller 2006, Ordovas 2006b). The nuclear receptor superfamily of transcription factors, with 48 members in the human genome, is the most important group of nutrient sensors. Numerous receptors in this superfamily bind nutrients and their metabolites. For example, retinoic acid receptor (RAR) binds retinoic acid, vitamin D receptor (VDR) binds vitamin D, and peroxisome proliferator-activated receptors (PPARs) bind fatty acids. By activating these receptors, nutrients are able to influence a wide array of specific genes and cellular functions.

Until recently, researchers could only look at a few pre-selected genes and single or simple groups of nutrients. The tools developed as a result of sequencing the human genome have enabled researchers to take a much broader view. Nutrigenomics has been introduced for the research on the interaction of the genome with its nutritional environment. This science has the potential for a fine-tuning of what foods and nutrients and how much an individual should eat to maintain an optimal health.

3 Nutrigenomics and other 'omics technologies

3.1 Genome and genomics

The genome is the entire DNA sequence, the genetic fingerprint, of an organism. It contains all the nucleotide sequences including structural genes, regulatory sequences and non-coding DNA sequences (the Greek suffix 'ome' means 'complete' or 'all'). The Human Genome Project was a worldwide effort to clone, map and sequence the entire human genome and it is the largest ever international collaboration in biology. This task was finished in 2003 with the publication of the

complete human sequence (http://www.ornl.gov/sci/techresources/Human_Genome/home.shtml). The human genome is estimated to encode up to 30 000 genes, and to be responsible for generating more than 100 000 functionally distinct proteins (Trujillo et al 2006). The functions of many thousands of genes are not yet known.

Genomics is the study of the genome; an approach of mapping, sequencing, and analysis of all genes present in the genome, focusing on resolving the variation in the genome between individuals.

The sequencing of the human and other genomes has led to the development of a whole new scientific methodology. These new areas of scientific study usually include the 'omics' suffix. The technical developments have given us novel tools enabling high-throughput genome-wide approaches, i.e. covering the entire human genome. These tools form the basis of the biomics era; genomics (covering DNA), transcriptomics (RNA), proteomics (protein), metabolomics (metabolites) and systems biology (integrating all of these), with bioinformatics enabling the storage, integration and analysis of the overwhelmingly complex data set produced.

Genomics, genotyping, transcriptomics, proteomics and metabolomics, together with bioinformatics, constitute the discipline *functional genomics*, which is also referred to as *systems biology*. Functional genomics aims to uncover both the functional roles of different genes and how these genes interact with, and/or influence, each other in the functional network underlying health and disease.

With the completion of the Human Genome Project, it was realized that a new era in biological and medical sciences was beginning. This is often referred to as the 'omics' revolution (Mariman 2006). New technologies and knowledge from the Human Genome Project were combined with those of established scientific disciplines such as pharmacology and toxicology leading to the terms 'pharmacogenomics' and 'toxicogenomics'. Accordingly, the introduction of genomics approaches in nutritional sciences lead to the scientific area called 'nutrigenomics'.

3.2 Nutrigenomics and nutrigenetics

Nutritional genomics, or nutrigenomics, is the study of how food and genes interact and aims to understand the effects of diet on an individual's genes and health. It attempts to study the genome-wide influences of nutrition and identify the genes that influence the risk of diet-related diseases on a genome-wide scale, and to understand the mechanisms that underlie these genetic predispositions (Müller & Kersten 2003).

Nutrigenomics aims to determine the influence of common dietary ingredients on the genome, and attempts to relate the resulting different phenotypes to differences in the cellular and/or genetic response of the biological system (Mutch et al 2005). More practically, nutrigenomics describes the use of functional genomic tools to probe a biological system following a nutritional stimulus that will permit an increased understanding of how nutritional molecules affect metabolic pathways and homeostatic control. *Nutrigenetics*, on the other hand, aims to understand how the genetic makeup of an individual coordinates their response to diet, and thus considers underlying genetic polymorphisms. It embodies the science of identifying and characterizing gene variants associated with differential responses to nutrients, and relating this variation to disease states. Therefore, both disciplines aim to unravel diet/genome interactions; however, their approaches and immediate goals are distinct. Nutrigenomics will unravel the optimal diet from within a series of nutritional

alternatives, whereas nutrigenetics will yield critically important information that will assist clinicians in identifying the optimal diet for a given individual, i.e. personalized nutrition.

3.3 Nutrigenomics: measuring nutrition-responsive genome activity

Nutrigenomics is the science that examines the response of individuals to dietary compounds, foods and diets using post-genomic and related technologies, i.e. genomics, transcriptomics, proteomics and metabolomics. It studies the genome-wide influences of nutrition or dietary compounds on the transcriptome, proteome and metabolome of cells, tissues or organism at a given time.

3.3.1 Transcriptomics

The transcriptome is the complete set of RNA that can be produced from the genome. Transcriptomics is the study of the transcriptome, i.e. gene expression at the level of the mRNA. Using either cDNA or oligonucleotide *microarray technology*, it describes the approach in which gene expression (mRNA) is analysed in a biological sample at a given time under specific conditions. It is the most widely used of the 'omics technologies.

Regulation of the rate of transcription of genes by food components represents an intriguing site for regulation of an individual's phenotype (Trujillo et al 2006). A host of essential nutrients and other bioactive food components can serve as important regulators of gene expression patterns. Macronutrients, vitamins, minerals, and various phytochemicals can modify gene transcription and translation, which can alter biological responses such as metabolism, cell growth, and differentiation, all of which are important in the disease process. Genome-wide monitoring of gene expression using DNA microarrays allows the simultaneous assessment of the transcription of thousands of genes and of their relative expression between normal cells and diseased cells or before and after exposure to different dietary components. This information should assist in the discovery of new biomarkers for disease diagnosis and prognosis prediction and of new therapeutic tools.

Microarrays have been described as "the hottest thing in biology and medicine since the advent of the polymerase chain reaction" (Page et al 2003). The technology emerged around 1996 and had its first high-profile uses in 1998 and 1999 (ref 3-5 in Page et al 2003). The best practices and standard operating procedures for microarray analyses in nutrigenomic studies have been defined by Garosi et al (2005).

Microarrays represent a powerful tool for studies of diet-gene interactions. Their use, however, is still associated with a number of technical challenges and potential pitfalls (Garosi et al 2005). The high costs of microarrays with the complex logistical issues associated with performing nutritional microarray studies often mean that compromises have to be made in the number and type of samples analysed. Technical variations between array platforms and analytical procedures will almost inevitably lead to differences in the transcriptional responses observed. Consequently, conflicting data may be produced, important effects may be missed and/or false leads generated. This is likely to be particularly true in the field of nutrition, in which we expect that many dietary bioactive agents at nutritionally relevant concentrations will elicit subtle changes in gene transcription that may be critically important in biological terms but will be difficult to detect reliably. Thus, great care should always be taken in designing and executing microarray studies.

A Japanese group (Saito et al 2005) has established a web-based, integrated database for the publications and microarray expression data in the field of nutrigenomics. It is available at <http://a->

yo5.ch.a.u-tokyo.ac.jp/index.phtml. The database was designed for effective storage, management, analysis and sharing of gene expression data to nutritional scientists involved in microarray experiments. All information in it is freely available. Currently, there are more than 400 publications and several expression data sets available for any user. The database is still under testing and construction.

3.3.2 Proteomics

Dietary components can also modify the translation of RNA to proteins and the posttranslational events, which can affect protein activity (Trujillo et al 2006). Just as the genome is the entire set of genes, the proteome is the set of proteins produced by a species. However, unlike the genome, the proteome is dynamic and varies according to the cell type and the functional state of the cell. The complexity of a proteome is overwhelming. While the human genome ‘only’ comprises > 25 000 genes, the human proteome is estimated to encompass several 100 000 proteins and an order of magnitude more protein forms and variants.

Proteomics is the study of the proteome, and it addresses three categories of biological interest: protein expression, structure and function (Kusmann et al 2006). It attempts to characterize all proteins in a biological sample, including their relative abundance, distribution, post-translational modifications, functions, and interactions with other biological molecules. Proteomics is technically very challenging and the presence/absence of protein is not necessarily indicative of metabolic change.

Currently, the most widely used technologies for proteomics are two-dimensional (2D) gel electrophoresis to separate the proteins in a complex mixture isolated from cells or tissues, and specialised mass spectrometry techniques as protein identification tools (Fuchs et al 2005, Harland 2005, Kusmann et al 2006, Mariman 2006, Wang et al 2006). This is a rapidly developing field, and new and improved techniques continue to emerge.

The potential value of proteomics for the nutritional sciences has been recognized for some years. However, in contrast to the techniques for large-scale transcriptome analysis that are already used by the nutrition research community and which have led to numerous published studies, only a very few reports have described the use of proteome analysis as a tool in nutrition research. Most of them have involved the use of rodent models or human cells in culture (Fuchs et al 2005).

3.3.3 Metabolomics

One of the newest “omics” technologies in nutrition is metabolomics. It focuses on the analysis of metabolites, the metabolome. It tries to measure the level of all substances (other than DNA, RNA or protein) present in a sample; the metabolome comprises the complete set of metabolites synthesized by a biological system (Corthésy-Theulaz et al 2005, Gibney et al 2005, Harland 2005). Such a system can be defined by level of biological organization, such as organism, organ, tissue, cell, or cell compartment levels. Biologically relevant samples can easily be obtained from blood, urine, saliva, and fecal water. Metabolomics is a useful tool for generating individual metabolite profiles, such as complete plasma lipid (ie, cholesterol, triglycerides) and vitamin profiles.

Metabolomics examines the whole metabolism, which ultimately reflects the behaviour of different patterns of genes. It investigates metabolic regulation and fluxes in individual cells or tissues, in response to specific environmental changes. In common with transcriptomics and proteomics it involves the non-targeted determination of all metabolites present under specific environmental

conditions. The analysis and interpretation of the data that is derived from the comparison of different cell conditions is achieved by the use of bioinformatics. Some researchers use the term metabolomics to refer to both simple (cellular) and complex (whole tissue or organism) systems, others distinguish between *metabolomics* studies that are simple system only and *metabonomics* in complex systems (Whitfield et al 2004, Harland 2005). In metabonomics, systematic biochemical profiles and regulation of function are determined in whole organisms by analysing biofluids and tissues.

Metabolomics is making great advances in this complex approach to nutrition research (German et al 2004, Whitfield et al 2004, Gibney et al 2005, Rochfort 2005). This is partly because nuclear magnetic resonance (NMR) and mass spectroscopy (MS) are established techniques, but also because the application of pattern-recognition statistics, such as principle component analysis, is conventional in this field. Metabolomics also has the advantages of offering more immediate information about our metabolism, which is not presented by changes in gene transcription or protein expression since both can occur without apparent metabolic consequences.

At the present time, there is a limited number of researchers with the facilities required to do such specific studies. Until now there are only few reported examples of metabolomics in human subjects. Most examples have involved the metabolic profiling of individuals, where large-scale analyses of body fluids have been used to diagnose for metabolic disorders or exposure to xenobiotics (Whitfield et al 2004, Harland 2005).

In contrast to transcriptomics, proteomics and metabolomics are not yet routine and standardized procedures. It is not yet possible to measure the whole proteome or metabolome, and it is not known how many endogenous metabolites exist or how many exogenous food-derived metabolites can be measured in human samples.

There is currently no single technology capable of comprehensively assessing all of the metabolites in a biological sample. Metabolomics continue to face challenges such as sample preparation, technological sensitivity, lack of standardized statistical methods and public databases. Nevertheless, their potential benefits for health management are undisputed and have fuelled current efforts to assess, utilize, interpret, and ultimately integrate these global technologies in order to define a phenotype characterizing health status.

Metabolomics will be central to biology in the coming decades because it has been highlighted for funding in the recently published roadmap of NIH <http://nihroadmap.nih.gov>. A major initiative of the roadmap is the construction of libraries of small molecules and their chemistry (Gibney et al 2005).

3.3.4 Systems biology

The long-term aim of nutrigenomics is to understand how the whole body responds to real foods using an integrated approach termed 'systems biology'.

Systems biology is an approach for studying biological systems that analyzes multiple macromolecular species (DNA polymorphisms, RNA, protein, metabolites, etc) in one experiment. It is a holistic approach to study biological systems.

In the relation between nutrition and health (unlike the relation between nutrition and disease) it is necessary to develop a new concept for *biomarkers* (van Ommen & Stierum 2002). The biomarker

needs to reflect subtle changes in homeostasis and the efforts of the body to maintain this homeostasis. Also, it should preferably notice a wide variety of biological actions. Furthermore, both efficacy and safety aspects should be monitored simultaneously. Single nutrients may have multiple known and unknown biochemical targets and physiological actions, which may not be easily addressed with classical biomarkers (i.e. the single-gene, protein or metabolite approach), usually under non-physiological conditions. In addition, the efficacy assessment of health effects of nutritional components is even further complicated by the fact that single dietary constituents are hardly consumed as separate entities, but are part of a dietary mixture.

An important challenge in the development of functional foods for the prevention of complex (multifactorial) diseases is to obtain a better and improved overview (holistic) picture of early phases of the process (van Ommen & Stierum 2002).

The concept of systems biology relates to the integration of all information at the different levels of genomic expression (mRNA, protein, metabolite). Thus, systems biology generates pathway information and provides the capacity to measure (small) perturbations of the pathway resulting from nutritional influences. The challenge here is not so much on a technological level, as enormous progress is being made in the 'omics' technologies. Instead, it is the bioinformatics side (data processing, clustering, dynamics, integration of the various omics levels, and so on) that will have to produce major breakthroughs in order for systems biology for nutrition to mature.

Nutrition is chronic, constantly varying, and composed of a very large amount of known and unknown bioactive compounds. Furthermore, nutrition touches the core of metabolism by supplying the vast majority of ingredients (both macro- and micronutrients) for maintaining metabolic homeostasis. Thus, nutrition by its nature *needs* to be studied in an integrated way (Corthésy-Theulaz et al 2005).

These analytical platforms are now widely used by both the pharmaceutical and nutritional communities alike; however, whereas pharmaceuticals have a targeted approach aimed at restoring health, diet is a multi-parametric approach to preserve and/or optimise health. Indeed, the diet is comprised of a multitude of nutritional and chemical molecules each capable of regulating disparate biological processes, and thus cannot use an approach similar to the pharmaceutical industry, i.e. 'one drug one target' paradigm. Hence, nutrition is a true integrative science that is well positioned to benefit from the exploitation of novel technologies capable of assessing biological networks rather than single endpoints (Mutch et al 2005). Despite the powerful analytical platforms available for the analysis of genes, proteins, and metabolites, few examples have used a comprehensive and integrative approach to understand the influences of nutritional factors on metabolism. Rather, the great majority of intervention studies performed to date are platform-specific. Inasmuch as each of these analytical platforms provides increasingly accurate information describing a given phenotype, it is the integration of these technologies that provides the optimal means to unravel the effects of a biological challenge on an organism; thus the concept of systems biology (or integrated metabolism). The integrated metabolism approach yields an attracting and exciting future for both pharmaceutical and nutritional communities, and their quest to ameliorate health and prevent disease.

Currently, few examples exist in which an integrated approach has been used to examine the influences of exogenous factors on metabolism (ref 22-24, 138, 139 in Mutch et al 2005); yet, even these eloquent examples have only provided the genomic and metabolomic profiles of a single organ. Nevertheless, the underlying theme begins to emerge with these studies.

3.4 Nutrigenetics: measuring interindividual response to nutrients

3.4.1 Single nucleotide polymorphisms (SNPs)

All humans are 99.9 % identical at the gene sequence level (Kaput & Rodriguez 2004). The 0.1 % variations in sequence, however, produce the differences in phenotypes (hair and skin colour, height, weight, etc.) and in an individual's susceptibility to disease and health. Alterations in phenotype result from differences in gene expression or altered macromolecular activities.

The human population is characterised by genome diversity due to the presence of many polymorphisms. Most genes have small sequence differences (polymorphisms) that occur between individuals (Trujillo et al 2006). The most common form of polymorphism is the single nucleotide polymorphism (SNP), i.e. single base variations within a DNA sequence; they make up about 90 % of all human genetic variation. Scientists have identified over 3 million locations where single-base DNA differences occur in humans and thus are potential sites for introducing variability. Some of these differences could affect the way one individual responds to nutritional environment relative to another individual. - The Single Nucleotide Polymorphism (SNP) Consortium is mapping important polymorphic sites within the genome that dictate individual phenotypic differences among the population (<http://ncbi.nif.gov/SNP>).

SNPs seem to be important in explaining some of the variations in response to food components. A number of SNPs are already known to have nutritional consequences, for example in relation to folate and lipoprotein metabolism (see 3.4.2). Specific genetic polymorphisms in human populations change their metabolic responses to diet and can have an important effect on disease risk. Inter-individual genetic variation is also likely to be a crucial determinant of differences in nutrient requirements (e.g. folate).

It is already apparent that there are many polymorphisms that influence risk of nutrition-related chronic diseases like cardiovascular disease and type 2 diabetes. SNP analysis provides a powerful molecular tool for investigating the role of nutrition in human health and disease, and their consideration in clinical, metabolic and epidemiological studies can contribute enormously to the definition of optimal diets (Mutch et al 2005).

3.4.2 Genetic responses to individual nutrients

Nutrigenetics is the science behind single gene / single food compound relationships. Currently we are aware of about 20 genes that have polymorphisms that appear to confer a significant disadvantage, which may be overcome with dietary modification. Businesses like Sciona Inc. (www.sciona.com) and Genelex Inc. (www.genelex.com) in the US base their services on this knowledge and the scientific literature that supports it (see **Appendix 3**).

One of the best-described examples is the relationship between folate and the gene for methyltetrahydrofolate reductase (MTHFR). MTHFR has a role in supplying 5-methylenetetrahydrofolate, which is necessary for the re-methylation of homocysteine to form methionine. Methionine is essential to many metabolic pathways including production of neurotransmitters and regulation of gene expression. Folate is essential to the efficient functioning of MTHFR.

There is a common polymorphism in the gene for MTHFR where thymine replaces cytosine at base pair 677, leading to two forms of the protein: the wild type (C), which functions normally, and the

thermal-labile version (T), which has a significantly reduced activity (Frosst et al 1995). People with two copies of the wild-type gene (CC) or one copy of each (CT) appear to have normal folate metabolism. Those with two copies of the unstable version (TT) and low folate intake, have higher plasma homocysteine levels, which increases their risk of cardiovascular disease and premature cognitive decline. Supplemented with folic acid or increased intake of folate from food sources, these individuals quickly metabolise the excess homocysteine restoring their methionine levels to normal. The beneficial effect of folic acid supplementation (1 mg/day for 3 months) on plasma homocysteine level has been shown in a genotype-stratified, randomized, double-blind, placebo-controlled trial (Miyaki et al 2005).

Other examples of the interrelationship between SNPs and specific food components include (Buttriss 2006, Ordovas 2006a, Trujillo et al 2006):

- Apolipoprotein E (apo E): Three isoforms of apo E protein are found in most populations: E2, E3 and E4. People with apo E4 genotype (typically about 25 % of the population) have a higher risk of cardiovascular disease and usually higher LDL cholesterol levels, and respond better to a low-fat diet. In addition, E4 allele results in an increase in LDL cholesterol in response to high-dose n-3 PUFA supplementation in subjects who already have abnormal blood lipid levels.
- Peroxisome proliferator-activated receptor gamma (PPAR γ): Subjects with a common variant of the gene (substitution of alanine for proline; 26 % of the population) have a higher BMI with a lower P:S ratio but a lower BMI if their diet has a higher P:S ratio.
- Several polymorphisms of vitamin D receptor (VDR) gene may affect the response to various dietary components (calcium, fat, vitamin D) and possibly disease risk. Caffeine may be a risk factor for bone loss in women with a certain variant of VDR and they may need to reduce the amount of caffeine in their diet to cut their risk of osteoporosis.
- A polymorphism in angiotensinogen gene may determine how an individual's blood pressure responds to dietary fibre.
- Glutathione peroxidase: Selenium may help prevent cancer in humans; however, all individuals may not respond equally. A polymorphism of human glutathione peroxidase has been associated with increased risk of several cancers.
- Alcohol dehydrogenase: Protective effect of moderate alcohol consumption in cardiovascular disease is greatest in people with a variant form of the alcohol dehydrogenase enzyme that leads to slower alcohol metabolism.

Also lactose intolerance is an excellent example of adverse genome-diet interactions. Lactose intolerance limits the use of fresh milk and milk products among adults and produces a painful digestive condition commonly associated with nausea, cramps, bloating, gas, and diarrhea.

Recently, investigators (Enattah et al 2002) have identified a genetic variant or SNP (C/T13910), 14kb upstream of the *LCH* (lactase-phlorizin hydrolase) locus on the large arm of human chromosome 2 (2q21). This variant, first identified in nine extended Finnish families, is responsible for lactose tolerance (lactase persistence), the ability of adults to consume fresh milk and milk products without complications. The polymorphism occurs in the promoter of the lactase gene and is thought to alter regulatory protein - DNA interactions controlling expression of the gene in adults. The 11 polymorphisms of this gene are clustered into 4 (A, B, C, U) prevalent (> 0.05%) haplotypes. The A haplotype conferring lactose tolerance has an 86% frequency in the northern European population, but only 36% in southern European populations. Cultures that drink fresh milk generally have a higher frequency of the A allele. The persistence of this gene in populations may confer selective advantages that include improved nutrition, prevention of dehydration, and improved calcium absorption.

The emergence of the lactase persistence variant is a relatively recent evolutionary event, appearing in Northern Europeans only 10,000 to 12,000 years ago (approximately with the time that animals were domesticated). The discovery of the C/T13910 DNA variant should now make it possible to design individualized dietary interventions based on a genetic test for lactose intolerance in early childhood.

4 Use of nutrigenomics approach in nutrition research

A literature survey was carried out to search for studies applying 'omics technologies for investigation of the effects of nutrients, phytochemicals, foods or diets either in cell models, experimental animals or humans. The studies using transcriptomics (microarray technology), proteomics and metabolomics are listed in **Appendix 1**.

4.1 Transcriptomics

There are several studies using microarray technology to investigate the effects of foods, food components or nutrition on gene expression in cell models, many studies in animals, and only few in humans. The first paper was published in 1999 in *Science*, describing the gene expression profile of the aging process in skeletal muscle of mice and its restriction by caloric restriction (Lee et al 1999).

4.1.1 Studies in cell models

Human cancer cell lines are the most common cell models employed in these studies, but also human or rodent adipocytes and some other cell types have been used. The following food components have been studied in cell cultures (for references, see 1.1 in **Appendix 1**):

- colon cancer cells: short-chain fatty acids, epicatechin and cocoa polyphenolic extract, apple flavonoids
- prostate cancer cells: soy isoflavones
- bladder tumor cells: genistein
- adipocytes: anthocyanins
- pancreatic cells: fatty acids
- neuroblastoma cells: retinoic acid
- osteoblastic cells: 1,25(OH)₂D₃

4.1.2 Animal studies

Experimental animals – in most cases mice – have been used to study the effects of caloric restriction, macronutrients, micronutrients, as well as phytochemicals, plant extracts or foods on gene expression in various tissues, for example (references in 1.2 in **Appendix 1**):

Ageing & caloric restriction (several studies)

- mouse, rat, rhesus monkey
- liver, skeletal muscle, heart, brain

Macronutrients

- high-fat diet: adipose tissue, liver, muscle
- high-fat diet and antioxidant supplement: muscle
- fish oil: liver, hippocampus

- protein (soy, whey, casein, gluten): liver, colon

Micronutrients

- low selenium status, depletion, supplementation
- zinc status
- magnesium deficiency
- α -tocopherol deficiency
- vitamin K

Phytochemicals, extracts, foods

- genistein: liver prostate cancer bone metastasis
- herbal extract (*Ginkgo biloba*): mouse brain
- vegetables: mouse colonic mucosa, lung
- cocoa: rat liver, mesenteric adipose tissue

4.1.3 Human studies

There are several microarray studies on gene expression profiling in human adipose tissue and a couple of studies in human skeletal muscle tissue (without dietary intervention), usually related to obesity (1.3.1 and 1.3.2 in **Appendix 1**). These studies have focused on visceral adipose tissue, or on comparison of subcutaneous and visceral adipose tissue, preadipocytes and adipocytes, or obese and non-obese subjects.

As far as we know, only four papers have so far been published on the use of microarray profiling in dietary intervention trials (1.3.3 in **Appendix 1**). Clement et al (2004) and Dahlman et al (2005) investigated the effects of energy-restricted diets on adipose tissue gene expression in obese subjects. Di Caro et al (2005) studied the influence of the probiotic *Lactobacillus GG* on gene expression pattern in small bowel mucosa. van Erk et al (2006) investigated the effects of high-carbohydrate and high-protein breakfasts on gene expression profile in blood cells. These studies are shortly described below. – The multicentre dietary interventions currently ongoing in Europe (see 6.2) focus on the effects of dietary fat modification in metabolic syndrome and on the impact of glycemic index and protein content of the diet in obesity and weight management. In the University of Kuopio, nutrigenomic approaches have been applied in four human interventions carried out at the Department of Clinical Nutrition and Food and Health Research Centre. In these studies the effects of weight reduction and physical activity as well as consumption of rye, berries and fish on gene expression in adipose tissue and blood peripheral mononuclear cells have been studied. The first manuscripts have been sent for the review.

A weight loss intervention trial carried out in France investigated the effects on inflammation-related genes in adipose tissue of obese subjects (Clement et al 2004). Gene expression profiles of subcutaneous white adipose tissue were analysed from 29 obese subjects during very low calorie diet (VLCD) using cDNA microarray and reverse transcription quantitative PCR. The patterns of expression were compared with that of 17 non-obese subjects. Gene expression profiling identified 100 inflammation-related transcripts that are regulated in obese individuals when eating a 28-day VLCD but not a 2-day VLCD. Cluster analysis showed that the pattern of gene expression in obese subjects after 28-day VLCD was closer to the profile of lean subjects than to the pattern of obese subjects before VLCD. Weight loss improved the inflammatory profile of obese subjects through a decrease of proinflammatory factors and an increase of anti-inflammatory molecules. The genes were expressed mostly in the stromavascular fraction of adipose tissue, which was shown to contain numerous macrophages. The authors concluded that the beneficial effect of weight loss on obesity-related complications may be associated with the modification of the inflammatory profile in adipose tissue.

The other trial was carried out in Sweden and studied the changes in adipose tissue gene expression with different energy-restricted diets in obese women (Dahlman et al 2005). Forty obese women were randomly assigned to a moderate-fat, moderate-carbohydrate diet or a low-fat, high-carbohydrate hypoenergetic (-600 kcal/d) diet for 10 wk. Subcutaneous adipose tissue samples were obtained before and after the diet period. High-quality RNA samples were obtained from 23 women at both time points, and these samples were hybridized to microarrays containing the 8500 most extensively described human genes. The results were confirmed by separate messenger RNA measurements. Both diets resulted in weight losses of approximately 7.5% of baseline body weight. A total of 52 genes were significantly up-regulated and 44 were down-regulated as a result of the intervention, and no diet-specific effect was observed. No major effect on lipid-specific transcription factors or genes regulating signal transduction, lipolysis, or synthesis of acylglycerols was observed. Most changes were modest (<25% of baseline), but all genes regulating the formation of polyunsaturated fatty acids from acetyl-CoA and malonyl-CoA were markedly down-regulated (35-60% decrease). The authors concluded that macronutrients have a secondary role in changes in adipocyte gene expression after energy-restricted diets. The most striking alteration after energy restriction was a coordinated reduction in the expression of genes regulating the production of polyunsaturated fatty acids.

In Italy, Di Caro et al (2005) investigated the influence of *Lactobacillus GG* on the genetic expression patterns in the small bowel mucosa. Six male patients with endoscopically proven oesophagitis were enrolled. All patients were treated for 1 month with esomeprazole and randomised to receive *Lactobacillus GG* or placebo. After 1 month of treatment, upper endoscopy was repeated. Biopsies of the duodenal mucosa were taken prior to and after the treatment, and the genes expression patterns were assessed using GeneChip Human U133A array. Genes with significant expression changes were selected and analysed to identify specific cellular pathways modified by *Lactobacillus GG*. To support the array data, 10 target genes were studied using Syber-Green PCR. Microarray analysis showed that *Lactobacillus GG* administration determined the up- and down-regulation of 334 and 92 genes, respectively. Real-time PCR confirmed the reliability of the analysis. *Lactobacillus GG* mainly affected the expression of genes involved in immune response and inflammation (TGF-beta and TNF family members, cytokines, nitric oxide synthase 1, defensin alpha 1), apoptosis, cell growth and cell differentiation (cyclins and caspases, oncogenes), cell-cell signalling (ICAMs and integrins), cell adhesion (cadherins), signal transcription and transduction. The data indicated that administration of *Lactobacillus GG* is associated with a complex genetic response of the duodenal mucosa, reflected by the up- and down-regulation of several genes involved in specific cellular pathways.

A Dutch study (van Erk et al 2006) evaluated the potential of gene expression profiling in blood cells collected in a human intervention study that investigated the effect of a high-carbohydrate (HC) or a high-protein (HP) breakfast on satiety. Blood samples were taken from 8 healthy men before and 2 h after consumption of an HP or an HC breakfast. Both breakfasts contained acetaminophen for measuring the gastric emptying rate. Analysis of the transcriptome data focused on the effects of the HP or HC breakfast and of acetaminophen on blood leukocyte gene expression profiles. Breakfast consumption resulted in differentially expressed genes, 317 for the HC breakfast and 919 for the HP breakfast. Immune response and signal transduction, specifically T cell receptor signaling and nuclear transcription factor kappaB signaling, were the overrepresented functional groups in the set of 141 genes that were differentially expressed in response to both breakfasts. Consumption of the HC breakfast resulted in differential expression of glycogen metabolism genes, and consumption of the HP breakfast resulted in differential expression of genes involved in protein biosynthesis. Gene expression changes in blood leukocytes corresponded with and may be related to

the difference in macronutrient content of the breakfast, meal consumption as such, and acetaminophen exposure. This study illustrated the potential of gene expression profiling in blood to study the effects of dietary exposure in human intervention studies.

4.2 Proteomics

There are a few examples of the use of proteomics technology in nutrition research (section 2 in **Appendix 1**). The first paper was published in 2002.

Colon cancer cells

- butyrate
- flavone, quercetin

Endothelial cells

- genistein

Animals

- atherogenic diets: liver
- zinc deficiency: liver
- grape seed extract: brain

Humans

- obesity: skeletal muscle
- broccoli intervention: serum.

4.3 Metabolomics

Since 2003, a few papers have been published on the use of metabolomics strategy in nutrition research (section 3 in **Appendix 1**), for example:

Animals

- obese vs normal animals: urine
- epicatechin: urine

Humans

- serum fatty acid profiles in type 2 diabetic patients and healthy controls
- plasma phospholipid profiles in type 2 diabetes
- soy (isoflavone) intervention: plasma lipoprotein, amino acid and carbohydrate profiles
- chamomile tea intervention: urine
- metabolic effects of green and black tea consumption: urine, plasma

5 Academic centres and networks

5.1 National academic centres and consortia

Nutrigenomics research is being conducted in individual laboratories, multidisciplinary centres, departments, institutes and companies (**Appendix 2** and **3**). Many of these initiatives have specific goals or missions. Examples include: metabolic stress at the Nutrigenomics Consortium in the Netherlands; fatty acids in metabolic syndrome at the Institute of Molecular Medicine in Ireland; the biological basis of health disparities at the Center of Excellence in Nutritional Genomics at the University of California Davis; cause and control of inflammatory bowel diseases at the New Zealand Centre of Excellence in Nutrigenomics; and study of cardiovascular disease at Nutrition and Genomics Laboratory at Tufts University.

The only nutrigenomics organization whose mission is multinational and not explicitly linked to a specific research program is The European Nutrigenomics Organisation NuGO.

5.2 NuGO - The European Nutrigenomics Organisation

NuGO - The European Nutrigenomics Organisation - linking genomics, nutrition and health research (www.nugo.org) is a network of excellence funded through the European Union Sixth Framework Programme for Research and Technology and started in January 2004. Funding (17 300 000 €) will finish in December 2009 but NuGO is anticipated to continue in one form or another. The network is coordinated by Wageningen University and the director is Dr Ben van Ommen from TNO Nutrition and Food Research. Initially, the network comprised 22 collaborators but it is open to new members throughout its six-year term.

NuGO aims to integrate and develop nutritional genomics in Europe. Nutrition and health research is focussed on the prevention of disease by optimising and maintaining cellular, tissue, organ and whole-body homeostasis. This requires understanding, and ultimately regulating, a multitude of nutrient-related interactions at the gene, protein and metabolic levels. This network will enable nutrition research to fully complement the biomedical and pharmacological research communities that are currently using genomics for the development of curative therapy. A key objective of the network will be the development, data warehousing and exploitation of nutrition and health-related bioinformatics for the benefit of European nutrition researchers, and for the community as a whole.

As a network of excellence, NuGO does not do research as such. Its purpose is to overcome the fragmentation of research, giving researchers from across Europe the chance to work together, share facilities and their expertise. The main deliverable is a durable structure and clear strategy for future research.

The following 23 partner organisations from 10 European countries form the core of NuGO (partner coordinators are given in parentheses):

The Netherlands

Wageningen University (Michael Müller)

TNO Nutrition & Food Research (Ben van Ommen)

RIKILT – Institute of Food Safety (Jaap Keijer)

Maastricht University (Edvin Mariman)

RIVM - National Institute of Public Health & the Environment (Edith Feskens)

Topshare International B.V., Wageningen (Marten Renkema), SME partner

Ireland

University College Cork (Albert Flynn)

Trinity College Dublin (Helen Roche)

University of Ulster (Coleraine) (Sean Strain)

Germany

German Institute of Human Nutrition (DIfE) (Judith Schäfer)

Technische Universität München (Hannelore Daniel)

Sciencion AG, SME partner

UK

Institute of Food Research, Norwich (Siân Astley)

Rowett Research Institute, Aberdeen (Harry J. McArdle)

University of Reading - Centre for Integrated Nutritional Genomics (Jenny Ames)

University of Newcastle (John Mathers)

European Bioinformatics Institute, Cambridge (Susanna Asunta Sansone)

Italy

University of Firenze, Department of Preclinical and Clinical Pharmacology (Piero Dolara)

Poland

The Jagiellonian University Medical College (Aldona Dembinska-Kiec)

Sweden

Lund University (Björn Åkesson)

Spain

University of the Balearic Islands (Andreu Palou)

France

National Institute of Health and Medical Research – INSERM (Denis Lairon)

Norway

University of Oslo, Institute of Nutrition Research (Cristian A. Drevon, Hege Berg Henriksen)

The primary aims of NuGO are to:

- Train a new generation of European scientists to use post-genomic technologies in nutrition research
- Develop and integrate genomic technologies for the benefit of European nutritional science
- Facilitate the application of these technologies in nutritional research world-wide
- Create the world-leading virtual centre of excellence in nutrigenomics

The specific aims are to:

- Strengthen the European scientific and technological excellence in nutrigenomics by bringing together the critical mass of resources and expertise needed to offer leadership in this rapidly developing field
- Define individual response to nutrients and refine the requirements for population sub-groups based on genetic variation (nutrigenetics), sex, and the different life-stages
- Determine the relative health benefits and risks of food compounds for different population subgroups and improve public health
- Spread excellence in nutrigenomics beyond the partnership through training, sharing of methods and facilities, dissemination and exploitation, and enter into dialogue with stakeholder groups
- Support the competitive arm of the European food industry, facilitating its growth as a knowledge-based business, with a view to evidence-based healthier food production
- Promote understanding in the ethical, social, legal, economical and scientific issues of concern in nutrigenomics

There are four related activities, each with complementary tasks which are grouped into work packages (WP):

- *Joint Research Activities*, which will exploit the technological and scientific innovations in a number of key areas of nutrition and health research as well as consolidate the research effort in Europe
 - WP 1 Gut health and function
 - WP 2 Metabolic health
 - WP 3 Life stage nutrition
 - WP 4 Risk benefit evaluation
- *Integrating Activities*, which will harmonise, stimulate and facilitate new technologies, informatics and systems for common use, and underpin research activities
 - WP 5 Nutrigenomics technology standardisation and innovation
 - WP 6 Genomics methods for nutritional studies in humans and model systems

- WP 7 Bioinformatics and nutritional systems biology
- WP 8 Information systems and databases
- *Spreading of Excellence*, which will build upon the acquired knowledge, sharing it with stakeholder groups including researchers, industry, society and healthcare
 - WP 9 Training
 - WP 10 Communication
 - WP 11 Nutrigenomics and society
 - WP 12 Commercialisation
 - WP 15 Recruitment and management of small–medium sized enterprises (SME) interaction
- *Management*, coordinates and directs the network

For researchers, NuGO offers international events, information and advice about integrating post-genomic technologies into research and an opportunity to be involved with people at the forefront of this revolution in nutritional sciences. It is fostering collaborations among members through targeted funding and an interactive website, Nutrigenomics Information Portal www.nugo.org, which hosts discussion groups on subjects related to nutrigenomics research methods and results. The website has a ‘public’ part and a ‘registrants only’ intranet part. Registration is free and includes membership of the Nutrigenomics Society. The intranet part contains more information and allows interaction and contribution to this website.

NuGO also wants to share its scientific knowledge with SMEs in support of the European Industry in the food, nutrition and biotechnology sectors. The only requirements are that the company is a small & medium sized enterprise (SME) and that it can match NuGO funding with its own money on a 1:1 basis. Topshare was the first SME involved in NuGO.

5.3 International Nutrigenomic Network

The complexity of nutrient-gene interactions has led to the realization that strategic international alliances are needed to improve the completeness of nutrigenomic studies - a task beyond the capabilities of a single laboratory team.

The International Nutrigenomic Network was initiated in October 2004 at a meeting following the Bruce Ames International Symposium on Nutritional Genomics by members of the NCMHD Center of Excellence in Nutritional Genomics at the University of California Davis, the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Nutrigenomics New Zealand, and NuGO. Eighty-eight researchers from 22 countries have outlined the issues and challenges for harnessing the nutritional genomics for public and personal health (Kaput et al 2005, 2006). University of Kuopio is represented by Kaisa Poutanen. The goal of the network is to foster research in the nutrigenomic area, and at least for the initial period (and perhaps longer), to develop the network without bureaucracy.

The next step in the process of forming productive international alliances is the development of a virtual centre for organizing collaborations and communications that foster resources sharing, best practices improvements, and creation of databases. There are already plans and initial efforts of creating the Nutrigenomics Information Portal, a web-based resource for the international nutrigenomics society (Kaput et al 2006). This portal aims at becoming the prime source of information and interaction for nutrigenomics scientists through a collaborative effort.

Currently, there are at least two websites providing information on nutrigenomics. In addition to the web site of NuGO www.nugo.org (see 5.2), useful information and e-mail service are available

through the website of The Center of Excellence in Nutritional Genomics (University of California Davis) at [http://nutrigenomics.ucdavis.edu/ Information/Concepts in Nutrigenomics](http://nutrigenomics.ucdavis.edu/Information/Concepts%20in%20Nutrigenomics). The Center sends out abstracts, news media and government reports, editorials and commentaries from scientific journals with a list serve. The list serve was initiated in July 2002 to alert interested readers in nutrigenomics or topics related to the field. It was designed for a relatively small group (~100) of scientists to exchange comments by email about experiments and results. It was soon discovered that many scientists and individuals in lay public signed up for the emails. The list serve now serves over 1250 individuals in over 53 countries. Subscribing to the Nutrigenomics "News & Views" is free and it provides the latest news, information and commentary from some of the world's leading experts in the field of nutritional genomics.

6 Nutrigenomics in EU programmes

Five research projects with elements of nutrigenomics were funded in the Fifth framework programme (1998-2002) and five projects are currently being funded in the Sixth framework programme (2003-2006), see **Appendix 4**. The research mainly focuses on the effects of dietary fats (especially n-3 PUFA) or variations in carbohydrate glycemic index and protein content in obesity and metabolic syndrome in multicentre interventions.

6.1 Fifth framework programme

NUGENOB - Nutrient-gene interactions in human obesity: implications for dietary guidelines project was coordinated by Copenhagen University Hospital. The objective was to improve understanding of the role of interaction between nutrition, especially fat intake, and genetic variations in obesity, which may be the basis for revision of dietary guidelines. Obese and lean subjects from eight European cities (Copenhagen, Maastricht, Stockholm, Nottingham, Prague, Paris, Pamplona and Toulouse) had a high-fat test meal and measures of energy expenditure and fat oxidation were obtained before and after the meal, together with measures of the concentration of metabolites and hormones in the blood. The obese subjects underwent a 10-week weight loss randomised intervention with either moderate or low fat content, but the same reduction in energy intake (600 kcal/day). In all subjects a blood sample was obtained for genotyping. In the subgroup of obese subjects the activity of specific genes in fat tissue were examined before and after the weight loss intervention.

Summary of the major results (From: D'Amario & de Froidmont-Görtz 2005): The dietary fat energy percent was 25% in the low-fat group and 40% in the high-fat group, and the average weight loss was 6.9 and 6.6 kg in these groups, respectively. Dropout was higher in the high-fat group than in the low-fat group (18% versus 14%). Among completers, more subjects lost >10% in the low than in the high-fat group (21% versus 15%). Fasting plasma total, LDL, and HDL cholesterol decreased in both groups, but more so in the low-fat group. Fasting plasma insulin and glucose were lowered equally by both diets. Levels of mRNA of 10 out of the 38 genes, investigated in subcutaneous adipose tissue in a subset of patients examined before and after the diets in two groups, were regulated by energy restriction, but none of the genes showed a different response to the diets. Clustering analysis revealed new potential co-regulation of genes. In another series, adipose tissue mRNA samples were hybridized to microarrays containing the 8500 most extensively described human genes. A total of 52 genes were significantly up-regulated and 44 were down-regulated as a result of the intervention, and no diet-specific effects were observed. Thus, macronutrients may have a secondary role in changes in adipocyte gene expression after energy-restricted diets. A number of obesity-related candidate genes have been identified and genotyped for

potentially important genetic variation and analysis of their role in the diet-induced weight change is currently being analysed.

The project *DIET AND OBESITY - Dietary and genetic influences on susceptibility or resistance to weight gain on a high fat diet* was coordinated by Rowett Research Institute. The objective was to identify the causes of weight gain in the human population on a Western, energy dense, high-fat diet through description of genetic background and phenotypic traits (behavioural, metabolic, physiological, psychological) associated with dietary preference and relative susceptibility or resistance to weight gain and obesity. Studies of the mechanisms underlying susceptibility to diet-induced obesity (DIO), and responses to fattening diets utilised a rodent model. The project also addressed the effect of early life nutrition on dietary preferences, susceptibility to obesity, and activity of hypothalamic neuroendocrine systems involved in energy balance.

Summary of the major results (From: D'Amario & de Froidmont-Görtz 2005): The prevalence of high-fat (HF) and low-fat (LF) consumers was established, and phenotypes associated with susceptibility to weight gain and obesity were characterised. DIO-resistant and -susceptible phenotypes were rare within the sample population, with a prevalence of 2-5%. Characterisation involved assessment of attitudes, diet and lifestyle, physical, non-invasive measures, heart rate, activity, motivation to eat, and indirect calorimetry (energy and nutrient metabolism). Subjects susceptible to DIO were older, more restrained, and showed greater hedonic responses to foods, and a weaker effect on satiety when fed a HF diet. Susceptible individuals appeared to be aware and concerned about weight gain. The relatively infrequent HF consumer who is susceptible or resistant to DIO sits at the extreme end of a normal distribution. The number of individuals for whom detailed dietary information could be obtained, and to whom a clear phenotype could be ascribed, was restricted, resulting in small sample sizes that limited the power of molecular genetic candidate gene studies investigating the involvement of genetic variants in food preferences and susceptibility to obesity on a HF diet. The rat model of DIO exhibited a normally distributed range of body weight trajectories when fed a high-energy diet, making the model a good representation of the majority of human, which is multifactorial and polygenic in nature. The quality of nutrition in early life (the diet ingested by mothers during pre- and early postnatal periods) was important for the development of signalling pathways participating in the regulation of feeding behaviour. These effects may be long-lasting or permanent, including enhancement of the obesogenic potential of diets presented in adult life. The critical period for nutritional influence on key hypothalamic systems appeared to be gestation rather than lactation.

6.2 Sixth framework programme

NuGO - The European Nutrigenomics Organisation is one of the projects of the Sixth framework programme. This project is described above in section 5.2.

LIPGENE - Diet, genomics and the metabolic syndrome: An integrated nutrition, agro-food, social and economic analysis is coordinated by Institute of Molecular Medicine and Department of Clinical Medicine, Trinity College Dublin. It is a consortium of 25 research laboratories across Europe (Finnish partner: MTT Agrifood Research Finland, Animal Production Research / Animal Nutrition, Aila Vanhatalo). The research areas include human nutrition, genetics and metabolism, animal sciences, plant biotechnology, economics and consumer sciences. The project incorporates a human intervention trial in eight EU cities. Questions being addressed include:

- Can dietary fat quality improve insulin resistance in subjects with risk factors for metabolic syndrome?
- Do common gene polymorphisms influence response to dietary fat?

- How can changes in food chain fats be achieved?
- Can plant biotechnology provide a sustainable source of PUFA?
- What economic and consumer acceptability barriers exist?

The focus is the interaction between dietary fat composition and genotype in metabolic syndrome. Access to a large prospective cohort, together with clinical and nutritional data, provides a unique opportunity to determine diet-gene interaction in the development of metabolic syndrome. A large multi-centre dietary intervention study will examine responsiveness to dietary fat modification, varying both in fat content and composition, influence on insulin sensitivity and other aspects of metabolic syndrome. A key aim of is to improve the fat composition of milk and meat products (animal nutrition) and to provide novel sources of fatty acids (plant biotechnology). An economic analysis of the costs associated to metabolic syndrome will be undertaken, while consumer attitudes will be analysed.

DIOGENES - Diet, Obesity and Genes is coordinated by University of Maastricht. It is a pan-European programme targeting the obesity problem from a dietary perspective: seeking new insights and new routes to prevention. It is a consortium of 29 partners across Europe (Finnish partner: University of Helsinki, Department of Public Health, Jaakko Kaprio). It also includes 3 major food industrials and 5 small- and medium-sized enterprises.

DIOGENES aims to contribute to a better understanding of the obesity and its alleviation by dietary means by including all disciplines and stakeholders. To examine critically the dietary impact, an 8-country, long-term randomised, parallel dietary intervention study (diets with different glycemic index/protein levels) on whole families will take place. Food technology studies will seek to develop foods combining high consumer liking/preference with enhanced satiety signals to limit intake. Based on the new knowledge generated, the project will demonstrate prototypes of innovative products or advice regimens which will help susceptible individuals to avoid weight gain and re-gain, and link with implementers to facilitate commercialisation in the market place.

NUCSYS - Systems biology of nuclear receptors: A nutrigenomics approach to aging-related diseases is a Marie Curie Research Training Network coordinated by Department of Biochemistry, University of Kuopio. The network has 14 partners (Finnish partner: University of Oulu, Department of Pharmacology and Toxicology; Jukka Hakkola and Olavi Pelkonen).

The aim of this network is to meet two major challenges within the EC. Firstly, to dissect and exploit mechanisms, which relate dietary input to health, and secondly to train scientists in an interdisciplinary and intersectorial fusion of post-genomic arenas. Nutrigenomics aims to dissect mechanisms conjoining dietary factors to physiological function and pathological syndromes, such as cancer and osteoporosis. These and other aging-related syndromes remain poorly understood, but it is agreed that diet impacts both their incidence and prevention. Central to the body's ability to perceive and respond to dietary signals is the nuclear receptor superfamily of ligand-activated transcription factors, which respond to a range of macro- and micronutrients. Thus nuclear receptors act as nutrient sensors and play a central role in the dietary effects on the genome during aging and disease development. To unite the actions of multiple signalling pathways, NUCSYS will combine intersectorial perspectives of nuclear receptor biology and apply interdisciplinary expertise. Critical amongst these will be the application of systems biology skills to comprehensively model actions. Systems biology has emerged as a key mathematical approach to generate a unified paradigm for predicting the multitude of processes in a living cell. Key to realizing the originality and pertinence of the research objectives is the 'flow through' between partners of activities, personnel, reagents and expertise, which in turn is ensured by the delivery of the training objectives. The collected

technical expertise in the network ranges from modelling and bioinformatics to creation of inducible gene knockouts in mice and covers the most important areas of modern biosciences. The divergent partner institutions, ranging from universities and research institutes to commercial settings, including a SME, will provide an ideal ground for a broad practical and theoretical training of early stage researchers in the field of systems biology and nutrigenomics.

6.3 Seventh framework programme

The European Commission's main proposal for FP7 was published in April 2006, to be followed by its proposal of specific programmes in September 2006. Food, Biotechnology and Agriculture is one of the nine co-operative programs, where "Fork to Farm; Health and Well-being" is one of the themes. It is for sure that the nutrigenomics approach will be further supported in several of the research projects of the 7th framework program 2007-2013.

7 Nutrigenomics activities in Kuopio Science Park

In Kuopio, the long tradition of research into nutrient-gene interactions in the Department of Clinical Nutrition formed a natural basis for applications of nutrigenomics methodology in the beginning of the 2000s'. The Tekes project "Functional genomics and nutrition, FUNGENUT" started in 2003, and soon thereafter the project "Gene expression in obesity and insulin resistance, GENOBIN". Also in other departments the interest gradually increased, and another Tekes project "Molecular Nutrigenomics Technologies" started in 2004. This led to the first collaboration discussions, realised also in the workshop "Kuopio nutrigenomics platform", arranged by Carsten Carlberg and Kaisa Poutanen on 6 September 2004. Also the Agrobiotechnology Centre of Expertise at Technology Centre Teknia became involved, and published a leaflet "Kuopio Nutrigenomics Centre – Focusing on Genomics, Nutrition and Human Health" in 2005 (http://www.teknia.fi/upload/Nutrigenomics_PDF.pdf). Teknia also arranged a seminar on nutrigenomics in its "Knowledge from the top" seminar series on 30 August 2005, with two guest speakers from the international nutrigenomics centres, Prof. Sander Kersten (University of Wageningen, The Netherlands), and PhD Kevin Dawson (University of California, Davis, USA).

During the preparation of the current report, a questionnaire was sent out to the above mentioned and some other working groups and companies at Kuopio campus in order to gather the current activities and interests in the field. The groups were also invited to a seminar held on 28 August 2006.

8 Future prospects

8.1 Nutrition science and education

Dietary recommendations are based on epidemiological, clinical and experimental studies that have investigated biochemical, physiological and anthropometric outcomes of various populations. During the past two-three decades the development of new non-invasive or mildly invasive methods has been assisting in studying mechanisms in humans. Recent years have also brought nutrigenetics as part of the acknowledged nutritional research, and made it evident that nutrients and other bioactive compounds in food interact with genetic factors, which either modify the effects of these compounds or determine the way of function they have. This interplay is based on individual genetic make-up, and development of 'omics' methods has opened an avenue for studying it.

Systems biology or nutrigenomics – in spite of its name – aims to understand physiology and disease by integrating and considering molecular pathways, regulatory networks, cells, tissues, organs, and ultimately the whole organism.

How will this new discipline then benefit the nutrition science? According to Kaput et al (2005) nutrigenomic approach will yield short- and long-term benefits to human health by revealing novel nutrient-gene interactions, developing new diagnostic tests for adverse responses to diets, and by identifying specific populations with special nutrient needs. Mariman et al (2006) suggested the following deliverables: (i) New biomarkers for nutrition-related diseases. The comparison of biopsy material between patients and matched controls will reveal genes that are specifically up- or down-regulated during disease progression. Some of those genes or their combined expression profile can be used as biomarkers for different stages of disease. They can be incorporated into diagnostic protocols for determining the right moment for disease-stage-specific nutritional intervention or therapy. (ii) Biomarkers to monitor the efficacy of nutritional intervention. Similar comparative studies before, during and after an intervention will reveal genes and expression profiles indicative of the progress and the success of treatment by nutrition. (iii) Genes and molecular pathways as targets for prevention. Knowledge about the genes, the molecular pathways that they are part of and their specific role in the pathogenesis will bring forth novel strategies for prevention of disease or disease progress.

In short, nutrigenomics is expected to

- deliver biomarkers for health
- deliver early biomarkers for disease disposition
- differentiate dietary responders from non-responders
- discover bioactive food components.

There are enormous opportunities in nutrigenomics. It demands, however, interaction between different disciplines, such as biochemistry, bioinformatics, molecular biology, genetics and nutrition. Increasing understanding of molecular processes and dietary effects on physiological functions and health will eventually emphasize the need of training the undergraduate and graduate students in the field of nutrigenomics. In undergraduate program, molecular biology and associated courses in biochemistry and physiology form the basis of this training. Moreover, the terminology on nutrigenomics needs to be taught so that those graduating in clinical nutrition are able to read and understand scientific literature on modern nutrition research, increasingly involving nutrigenomics approaches to solve nutritional problems.

In graduate programs, current training can include nutrigenomics both in the theoretical course work and in research. The students can choose the courses quite freely, but there is a limited number of courses available in Kuopio. More emphasis should be put in the future in arrangement of relevant courses, also as a part of national and international graduate courses. The students should also be encouraged to complement their course portfolio to consist of the various elements of biochemistry, analytical methodologies, clinical nutrition and bioinformatics.

8.2 Commercial aspects & personalized nutrition

Personalized diets aiming at nutrition for maintenance of individual health and prevention of disease is often considered to be the major application outcome of nutrigenomics. Although significant advances have been made in understanding the relationship between dietary factors and disease prevention, the identification of those who will or will not benefit from dietary intervention

strategies remains a major obstacle to health promotion. Quick, low-cost, point-of-care tests are expected to assist patients and physicians to achieve, manage, and prolong health through dietary intervention.

In **Appendix 3**, nine companies in the field of nutrigenomics are presented. With the exception of one, they are all in the US, and actually are based on nutrigenetic rather than nutrigenomic data. The main business idea is to offer home genetic testing to consumers in order to assess the risk of certain chronic diseases, and to offer nutraceuticals, or dietary supplements, or personalized dietary advice, in order to minimize the risk of disease. These companies have already been criticized for being “at best ambiguous and at worst dangerous” (Nature vol. 442, 3.8.2006). It is clear that they are the very first generation attempts to commercialize nutrigenetic tests, and also offer rather simple solutions for risk reduction.

Science has great potential to increase understanding of the molecular mechanisms through which diet influences disease. However, currently the evidence to support clinical applications involving individualised dietary advice based on gene testing is not sufficient. Further, it is important to exercise caution in modifying nutritional messages aimed at the public as a whole as this would tend to confuse and dilute the message and would probably be detrimental to the population as a whole. We also would need a wide discussion concerning ethical issues of the use of nutrigenomics data, with involvement of scientists, but also civic organizations and authorities already in the beginning.

9 Literature

9.1 General papers and review articles on nutrigenomics

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APPENDIX 1. Studies using omics technologies in nutrition research

1 Transcriptomics, microarray studies

1.1 Studies using cell models

1.1.1 Human cancer cells:

Genetic reprogramming in pathways of colonic cell maturation induced by short chain fatty acids: comparison with trichostatin A, sulindac, and curcumin and implications for chemoprevention of colon cancer
Mariadason et al, *Cancer Res* 60, 4561-4572, 2000

Microarray profiling of gene expression patterns in bladder tumor cells treated with genistein
Chen et al, *J Biomed Sci* 8, 214-, 2001

Epicatechin and cocoa polyphenolic extract modulate gene expression in human Caco-2 cells
Noé et al, *J Nutr* 134, 2509-2516, 2004

Apple flavonoids inhibit growth of HT29 human colon cancer cells and modulate expression of genes involved in the biotransformation of xenobiotics
Veeriah et al, *Mol Carcinog* 45, 164-174, 2006

Soy isoflavones alter expression of genes associated with cancer progression, including interleukin-8, in androgen-independent PC-3 human prostate cancer cells
Handayani et al, *J Nutr* 136, 75-82, 2006

1.1.2 Adipocytes:

Gene expression profile of isolated rat adipocytes treated with anthocyanins
Tsuda et al, *Biochim Biophys Acta* 1733, 137-147, 2005

Microarray profiling of gene expression in human adipocytes in response to anthocyanins
Tsuda et al, *Biochem Pharmacol* 71, 1184-1197, 2006

1.1.3 Other cells:

The effect of chronic exposure to fatty acids on gene expression in clonal insulin-producing cells: studies using high density oligonucleotide microarray
Xiao et al, *Endocrinology* 142, 4777-4784, 2001

Detection of the retinoic-acid regulated genes in a RTBM1 neuroblastoma cell line using cDNA microarray
Ueda, *Kurume Med J* 48, 159-164, 2001

Microarray detection of gene expression changes induced by 1,25(OH)₂D₃ and a Ca²⁺ influx-activating analog in osteoblastic ROS 17/2.8 cells
Farach-Carson & Xu, *Steroids* 67, 467-470, 2002

1.2 Animal studies

1.2.1 Ageing & caloric restriction:

Gene expression profile of aging and its restriction by caloric restriction (mouse muscle)
Lee et al, *Science* 285, 1390-1393, 1999

Gene expression profile of the aging brain in mice caloric restriction
Lee et al, *Nat Genet* 25, 294-297, 2000

Genomic profiling of short- and long-term caloric restriction effects in the liver of aging mice
Cao et al, *PNAS* 98, 10630-10635, 2001

Transcriptional profiles associated with aging and middle age-onset caloric restriction in mouse hearts
Lee et al, PNAS 99, 14988-14993, 2002

Influence of aging and caloric restriction on the transcriptional profile of skeletal muscle from rhesus monkeys
Kayo et al, PNAS 98, 5093-5098, 2001

Effects of caloric restriction on mitochondrial function and gene transcripts in rat muscle
Sreekumar et al, Am J Physiol Endocrinol Metab 283, E38-43, 2002

1.2.2 Macronutrients:

Gene expression profile of rat adipose tissue at the onset of high-fat-diet obesity
Li et al, Am J Physiol Endocrinol Metabol 282, E1334-E1341, 2002

Dietary effects of arachidonate-rich fungal oil and fish oil on murine hepatic and hippocampal gene expression
Berger et al, Lipids Health Dis 1, 2, 2002

Impact of high-fat diet and antioxidant supplement on mitochondrial functions and gene transcripts in rat muscle
Sreekumar et al, Am J Physiol Endocrinol Metab 282, E1055-1061, 2002

Hepatic gene expression profiles in a long-term high-fat diet-induced obesity mouse model
Kim et al, Gene 340, 99-109, 2004

Genomic analysis of the response of mouse models to high-fat feeding shows a major role of nuclear receptors in the simultaneous regulation of lipid and inflammatory genes
Kreeft et al, Atherosclerosis 182, 249-257, 2005

High-fat feeding period affects gene expression in rat white adipose tissue
Lopez et al, Mol Cell Biochem 275, 109-115, 2005

Differentially abundant mRNAs in rat liver in response to diets containing soy protein isolate
Iqbal et al, Physiol Genom 11, 219-226, 2002

DNA microarray analyses of the effects of dietary proteins
Kato et al, Biofactors 21, 11-13, 2004

Dietary exposure to soy or whey proteins alters colonic global gene expression profiles during rat colon tumorigenesis
Xiao et al, Mol Cancer 4, 1, 2005

1.2.3 Micronutrients:

Gene expression profiling of low selenium status in the mouse intestine: transcriptional activation of genes linked to DNA damage, cell cycle control and oxidative stress
Rao et al, J Nutr 131, 3175-3181, 2001

Microarray analysis of selenium-depleted and selenium-supplemented mice
Hooven et al, Biol Trace Elem Res 109, 173-179, 2006

Changes in gene expression in rat thymocytes identified by cDNA array support the occurrence of oxidative stress in early magnesium deficiency
Petraut et al, Biochim Biophys Acta 1586, 92-98, 2002

Modulation of intestinal gene expression by dietary zinc status: effectiveness of cDNA arrays for expression profiling of a single nutrient deficiency
Blanchard et al, PNAS 98, 13507-13513, 2001

Gene expression profile of oxidant stress and neurodegeneration in transgenic mice deficient in alpha-tocopherol transfer protein
Gohil et al, Free Radic Biol Med 2003

Vitamin K suppresses lipopolysaccharide-induced inflammation in the rat
Ohsaki et al, Biosci Biotechnol Biochem 70, 926-932, 2006

1.2.4 Phytochemicals, extracts, foods:

Regulation of gene expression and inhibition of experimental prostate cancer bone metastasis by dietary genistein
Li et al, Neoplasia 6, 354-363, 2004

Hepatic gene expression profiles are altered by genistein supplementation in mice with diet-induced obesity
Kim et al, J Nutr 135, 33-41, 2005

Genomic responses to herbal extracts: lessons from in vitro and in vivo studies with an extract of *Ginkgo biloba* (mouse brain)
Gohil, Biochem Pharmacol 64, 913-, 2002

Vegetables affect the expression of genes involved in anticarcinogenic processes in the colonic mucosa of C57BL/6 female mice
van Breda et al, J Nutr 135, 1879-1888, 2005

Vegetables affect the expression of genes involved in carcinogenic and anticarcinogenic processes in the lungs of female C57BL/6 mice
van Breda et al, J Nutr 135, 2546-2552, 2005

Ingested cocoa can prevent high-fat diet-induced obesity by regulating the expression of genes for fatty acid metabolism
Matsui et al (Morinaga & Co, Ltd, Japan), Nutrition 21, 594-601, 2005

1.3 Human studies

1.3.1 Adipose tissue (without dietary intervention):

The gene expression profiling of human visceral adipose tissue and its secretory functions
Yang et al, Biochem Biophys Res Commun 24, 839-846, 2003

Gene expression profile of omental adipose tissue in human obesity
Gómez-Ambrosi et al, FASEB J 18, 215-217, 2004

Gene expression profiling in human preadipocytes and adipocytes by microarray analysis
Urs et al, J Nutr 134, 762-770, 2004

A survey of genes differentially expressed in subcutaneous and visceral adipose tissue in men
Vohl et al, Obes Res 12, 1217-1222, 2004

Differently expressed genes in visceral or subcutaneous adipose tissue of obese men and women
Linder et al, J Lipid Res 45, 148-154, 2004

Evidence of impaired adipogenesis in insulin resistance
Yang et al, Biochem Biophys Res Commun 317, 1045-1051, 2004

Obesity-related differential gene expression in the visceral adipose tissue
Baranova et al, Obesity Surgery 15, 758-765, 2005

Microarray profiling of isolated abdominal subcutaneous adipocytes from obese vs non-obese Pima Indians: increased expression of inflammation-related genes
Lee et al, Diabetologia 48, 1776-1783, 2005

Increased expression of inflammation-related genes in cultured preadipocytes/stromal vascular cells from obese compared with non-obese Pima Indians
Nair et al, Diabetologia 48, 1784-1788, 2005

A microarray search for genes predominantly expressed in human omental adipocytes: adipose tissue as a major production site of serum amyloid A
Sjöholm et al, J Clin Endocrinol Metab 90, 2233-2239, 2005

Downregulation of electron transport chain genes in visceral adipose tissue in type 2 diabetes independent of obesity and possibly involving tumor necrosis factor-alpha
Dahlman et al, Diabetes 55, 1792-1799, 2006

Microarray profiling of human adipose tissue after exogenous leptin injection
Taleb et al, Eur J Clin Invest 36, 153-163, 2006

1.3.2 Skeletal muscle tissue (without dietary intervention):

Microarray profiling of skeletal muscle tissues from equally obese, non-diabetic insulin-sensitive and insulin-resistant Pima Indians
Yang et al, Diabetologia 45, 1584-1593, 2002

Gene expression profile in skeletal muscle of type 2 diabetes and the effect of insulin treatment
Sreekumar et al, Diabetes 51, 1913-1920, 2002

1.3.3 Dietary interventions:

Adiponectin gene expression in subcutaneous adipose tissue of obese women in response to short-term very low calorie diet and refeeding (*not microarray*)
Liu et al, J Clin Endocrinol Metab 88, 5881-5886, 2003

Effects of different hypocaloric diets on protein secretion from adipose tissue of obese women (*not microarray*)
Arvidsson et al, Diabetes 53, 1966-1971, 2004

Adipose tissue gene expression in obese subjects during low-fat and high-fat hypocaloric diets (*not microarray*)
Viguerie et al, Diabetologia 48, 123-131, 2005

Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects
Clement et al, FASEB J 18, 1657-1669, 2004

Changes in adipose tissue gene expression with energy-restricted diets in obese women
Dahlman et al, Am J Clin Nutr 81, 1275-1285, 2005

Effects of Lactobacillus GG on genes expression pattern in small bowel mucosa
Di Caro et al, Dig Liver Dis 37: 320-329, 2005

High-protein and high-carbohydrate breakfasts differentially change the transcriptome of human blood cells
van Erk et al, Am J Clin Nutr 84: 1233-1241, 2006

2 Proteomics

2.1 Studies using cell models

Proteome analysis of butyrate-treated human colon cancer cells
Tan et al, Int J Cancer 98, 523-531, 2002
The first published paper describing the effects of a dietary compound using proteomics

Pleiotropic molecular effects of the pro-apoptotic dietary constituent flavone in human colon cancer cells identified by protein and mRNA expression profiling
Herzog et al, Proteomics 4, 2455-2464, 2004

Protein expression profiling identifies molecular targets of quercetin as a major dietary flavonoid in human colon cancer cells
Wenzel et al, Proteomics 4, 2160-2174, 2004

Proteome analysis for identification of target proteins of genistein in primary human endothelial cells stressed with oxidized LDL or homocysteine
Fuchs et al, Eur J Nutr 44, 95-104, 2005

2.2 Animal studies

Proteomics analysis of rat brain protein modulations by grape seed extract
Deshane et al, J Agric Food Chem 52, 7872-7883, 2004

Proteomic analysis of diet-induced hypercholesterolemic mice
Park et al, Proteomics 4, 514-523, 2004

Transcriptome and proteome analysis identifies the pathways that increase hepatic lipid accumulation in zinc-deficient rats
tom Dieck et al, J Nutr 135, 199-205, 2005

2.3 Human studies

Proteome analysis of skeletal muscle from obese and morbidly obese women
Hittel et al, Diabetes 54, 1283-1288, 2005

Evaluation of matrix-assisted laser desorption/ionization-time of flight mass spectrometry proteomic profiling: identification of alpha 2-HS glycoprotein B-chain as a biomarker of diet (broccoli intervention)
Mitchell et al, Proteomics 5, 2238-2246, 2005

3 Metabolomics

3.1 Animal studies

A combined ¹H NMR and HPLC-MS-based metabolomic study of urine from obese (fa/fa) Zucker and normal Wistar-derived rats
Williams et al, J Pharm Biomed Anal 38, 465-471, 2005

A rapid screening approach to metabolomics using UPLC and oa-TOF mass spectrometry: application to age, gender and diurnal variation in normal/Zucker obese rats and black, white and nude mice
Plumb et al, Analyst 130, 844-849, 2005

NMR-metabolomic studies on the biochemical effects of epicatechin in the rat
Solansky et al, J Agric Food Chem 51, 4139-4145, 2003

3.2 Human studies

Discrimination of type 2 diabetic patients from healthy controls using metabolomics method based on their serum fatty acid profiles
Yang et al, J Chromatogr B Anal Technol Biomed Life Sci 813, 53-58, 2004

Plasma phospholipid metabolic profiling and biomarkers of type 2 diabetes mellitus based on high-performance liquid chromatography / electrospray mass spectrometry and multivariate statistical analysis.
Wang et al, Anal Chem 77, 4108-4116, 2005

Application of biofluid ¹H nuclear magnetic resonance-based metabolomic techniques for the analysis of the biochemical effects of dietary isoflavones on human plasma profile
Solansky et al, Anal Biochem 323, 197-204, 2003

A metabolomic strategy for the detection of the metabolic effects of chamomile (*Matricaria recutita* L.) ingestion
Wang et al, J Agric Food Chem 53, 191-196, 2005

Metabolomics approach to determine metabolic differences between green tea and black tea consumption
van Dorsten et al, J Agric Food Chem 54, 6929-6938, 2006

APPENDIX 2. National academic centres and consortia for nutrigenomics research

Centre	Web site and contact person(s)	Mission
Nutrigenomics Consortium Netherlands Genomics Initiative THE NETHERLANDS	http://www.genomics.nl/Home/GenomicsNL/GenomicsCentres/Nutrigenomics.aspx Prof Michael Müller or Dr Inge Palm	Nutrigenomics Consortium is one of the 12 Genomics Centres of Netherlands Genomics Initiative. It aims to increase understanding of the events unfolding during metabolic stress, the early and reversible stage of metabolic syndrome. The ultimate objective is to discover and validate molecular biomarkers for the early detection of metabolic stress and to identify and develop novel food components for dietary management and prevention of metabolic stress. Wageningen University, Maastrich University and TNO Food and Nutrition Research are members of NuGO. - “Dietary signals, sensors and metabolic stress signatures”
Institute of Food Research UNITED KINGDOM	www.ifr.bbsrc.ac.uk	IFR's vision is to be a world-leading contributor to harnessing food for health and controlling food-related diseases. Many of its research programmes (e.g. Phytochemicals and Health, Micronutrients, Personalised Nutrition) include elements of nutrigenomics. IFR is member of NuGO.
Centre for Integrated Nutritional Genomics, University of Reading UNITED KINGDOM	http://www.food.rdg.ac.uk/ Prof Jenny Ames	University of Reading is a member organisation of NuGO through this Centre. It is working on the regulatory effects of fatty acids on molecular events involved in atherogenesis, including scavenger cell receptor regulation and gene events involved in fatty acid absorption in intestinal cells, including lipid raft structure and function. Maintenance of vascular health through diet is a major research priority using a range of endothelial cell systems to investigate normal and disturbed cell signalling events and their modulation at molecular level, by dietary constituents in vitro.
Institute of Molecular Medicine IRELAND	http://www.tcd.ie/IMM/research_nutrigenomics.php Dr Helen Roche	IMM is part of a research partnership in molecular medicine between Trinity College Dublin, University College Dublin and Royal College of Surgeons in Ireland. The Nutrigenomics Research Group is a partner in NuGO. Research topics: role of dietary fatty acids in cell signalling and gene expression events relevant to the metabolic syndrome and chronic pro-inflammatory diseases of the digestive system. The research spans from human nutrition intervention studies, through cell-line studies to conventional and transgenic animal studies and state-of-the-art genomic and transcriptomic facilities in all areas of molecular biology. Coordination of LIPGENE project.
BioProfile Nutrigenomics Berlin-Brandenburg GERMANY	www.kompetenznetze.de/vdi/generator/navi/en/Kompetenznetze/nutrigenomforschung.html Prof Hans-Georg Joost	Research groups from 5 universities, 10 SMEs, 1 major corporation, 4 non-university research institutes and 1 service provider are currently working on projects supported by the Nutrigenomics Network. Scientists from the Berlin-Brandenburg Nutrigenomics Network are active in NuGO.
Center of Nutrigenomics Technische Universität München GERMANY	http://www.nutrition.tum.de/en/index.php Prof Hannelore Daniel	TUM participates in NuGO via four research groups: <u>Human Biology</u> , <u>Nutrition Physiology</u> , <u>Biofunctionality of Food</u> , <u>Microbiology</u> as well as <u>Nutritional Medicine & Clinical Nutrition</u> . These groups also form the local <u>TUM-Nutrigenomics Center</u> . These research units aim to understand better human

		physiology and pathophysiology by employing molecular tools in cell culture systems, model organisms and in humans. Central to the research goals of these TUM units are processes that determine the bioavailability of nutrients and non-nutrient components of foods. By identifying, cloning and characterising the underlying transporter proteins in epithelial cells, the role of food compounds in human health can be better understood. The main compounds of interest are flavonoids. Effects of these ubiquitous compounds on epithelial and endothelial cell dynamics, transformation and apoptosis, and gene regulation are examined at the level of the cell and organs in model systems as well as cells obtained from human intervention trials.
Biocentrum-DTU (Danish Technical University) DENMARK	www.nutrigenomics.dtu.dk/English.aspx	The aim of the platform is to increase knowledge about the relationships between the genome, the functionality of food components, and food and health in food industry, health advice, and in the public. The food industry will gain access to solid scientific evidence for relationships between the genome, food, and health, which will support the development of products with a high nutritional quality and of functional foods. The Centre will contribute to making future health claims and labelling accurate and reliable, which will be an invaluable marketing asset. At the core of the research is the study of AMP-activated protein kinase (AMPK), which is believed to play an important part in the wholebody energy homeostasis and regulate important, metabolic pathways, such as e.g. hepatic ketogenesis, cholesterol synthesis and skeletal muscle fatty acid oxidation. Core bioinformatics competences include: four-dimensional understanding of the cell cycle, data warehouses of genome sequences, protein structures, and protein functions, including posttranslational protein modifications and protein-protein interactions.
Danish nutrigenomics platform DENMARK	Prof Lars Dragsted	The platform aims to be the forum of Danish public research, commercial research and stakeholders in the area of biomic nutrition research, and its relationship to food safety, quality, biotech and consumer attitudes. It aims to facilitate the convergence of food production, biotech and biomedical research and to utilise Danish strongholds in several of these areas to form an increased potential for Danish food innovation and international profiling. It focuses on energy metabolism and expenditure, pre- and probiotics, whole grain, fish, milk, meat and fruit bioactives.
Lund University Nutrigenomics SWEDEN	http://www.lunutr.lu.se/ Prof Björn Åkesson	Research and training in food and nutrition science is integrated within the <u>Lund Food Centre</u> with its <u>Functional Food Science Centre</u> . These centres represent an extensive and well-equipped facility for research in the nutrigenomics field integrating expertise in nutrition, food science, genomics and medicine. Selected researchers and doctoral students at these centres and departments, constituted in the working group LUNUTR, will participate in the joint programme of activities of NuGO and other programs.

<p>Kuopio Nutrigenomics Centre FINLAND</p>	<p>http://www.teknia.fi/upload/Nutrigenomics_PDF.pdf</p>	<p>Kuopio Nutrigenomics Centre, KNC, combines the University of Kuopio and the nearby University Hospital with the aim on studying lifestyle-gene interactions in well characterized human populations using modern molecular biology methods. The knowledge will be translated into tools of predictive diagnostics and personalized nutrition to retain human wellbeing. Moreover, the access to the Eastern Finland founder population provides a cost-effective way to access the impact of regulatory and functional single nucleotide polymorphisms (SNPs) on nutrition-induced signal transduction cascades. KNC's approach is to implement the new post-genomic techniques on genome, epigenome, transcriptome, proteome, metabolome and physiome function together with informatics and systems biology methodology in order to generate an integral view of these processes.</p>
<p>New Zealand Centre of Excellence in Nutrigenomics NEW ZEALAND</p>	<p>http://www.nutrigenomics.org.nz/ Prof Lynnette Ferguson</p>	<p>This is a strategic collaboration between <u>The University of Auckland</u>, <u>AgResearch Limited</u>, <u>HortResearch</u> and <u>Crop & Food Research</u>. The major aim is to determine how foods and food components affect health at the molecular genetic level by using nutritional genomic methods. This detailed understanding of the molecular mechanisms will ultimately lead to the development of completely new, added-value, export-focused, gene-specific foods that will deliver proven health outcomes to consumers. New Zealand food industry collaborators will deliver these foods to national and export markets. The initial focus is gut health, especially Crohn's disease. - "Tailoring New Zealand foods to match people's genes"</p>
<p>NCMHD Center of Excellence for Nutritional Genomics USA</p>	<p>http://nutrigenomics.ucdavis.edu/ > Information > Concepts in Nutrigenomics Prof Raymond L. Rodriquez</p>	<p>The Center is the result of a multi-disciplinary research collaboration between the <u>Section of Molecular and Cellular Biology</u> at the <u>University of California, Davis</u>, <u>USDA Western Human Nutrition Research Center</u>, <u>Children's Hospital of Oakland</u>, <u>Children's Hospital Oakland Research Institute (CHORI)</u>, and the <u>Ethnic Health Institute</u>. The mission is to reduce and ultimately eliminate racial and ethnic health disparities resulting from environment x gene interactions, particularly those involving dietary, economic, and cultural factors. The goal is to devise genome-based nutritional interventions to prevent, delay, and treat diseases such as asthma, obesity, Type 2 diabetes, cardiovascular disease, and prostate cancer. To achieve this goal, the Center is taking a multidisciplinary approach to develop culturally competent methods and novel technologies to elucidate the complex interactions between environmental triggers, genes, and disease.</p>
<p>Laboratory of Nutrigenomic Medicine (LNGM) @ University of Illinois Chicago USA</p>	<p>http://nutrigenomics.ucdavis.edu/ > Research > Concepts in Nutrigenomics > Resources > Academic Partnerships Prof Jim Kaput</p>	<p>LNGM is a translational research program focusing on nutrigenomics, nutrition, genetics, and clinical practice. LNGM has two primary aims (i) to study the association of variants of ~50 genes that others have linked to type 2 diabetes mellitus, with ~25 clinical measurements, and ~25 ingested nutrients, and (ii) develop information and knowledge for identifying patterns among diet, gene variants, and resulting physiologies in individual patients that can be used to improve diagnosis and treatments.</p>

<p>Nutrition and Genomics Laboratory HNRCA at Tufts University USA</p>	<p>http://hnrc.tufts.edu/departments/labs/genomics.php Prof Jose M. Ordovas</p>	<p>The Nutrition and Genomics Laboratory is a pioneer of the study of gene-diet interactions in the area of cardiovascular diseases, utilizing both genetic epidemiology approaches as well as controlled dietary intervention studies. Some highlights of this research include the elucidation of highly significant gene-diet interactions at several loci involved in lipid metabolism (e.g., APOE, LIPC, APOA1, APOA4, APOA5, PPARA) as well as in obesity (e.g., PLIN). In addition, it seeks to identify genes involved in longevity and healthy aging and to understand their regulation in response to dietary factors.</p>
<p>PSU Center of Excellence in Nutrigenomics, Penn State University USA</p>	<p>http://nutrigenomics.psu.edu/ Dr Jack Vanden Heuvel and Dr Penny Kris-Etherton</p>	<p>The mission is to enhance the use of molecular biology in basic and clinical nutrition studies. Research topics: the beneficial effects of diets high in alfa-linolenic acid, found enriched in walnuts.</p>
<p>University of Toronto, Department of Nutritional Sciences CANADA</p>	<p>http://www.utoronto.ca/nutrisci/ Prof Ahmed El-Soheby</p>	<p>Research Chair in Nutrigenomics The Canadian Nutrigenomics Committee has been created to identify Canadian strengths and key players in the field.</p>

APPENDIX 3. Companies in the field of nutrigenomics

Company	Web site	Mission	Products and services
AlphaGenics Rockville, Maryland, USA	http://www.alpha-genics.com	Uses genetics to develop personalized consumer lifestyle products. The target markets include personal performance, security, vanity, personal relationships, and personal comfort. The vision is that people will buy gene-based products that let them feel better about themselves and make them happier. The focus is on the positive parts of people's lives, not disease ('normal' genes instead of disease-related genes).	JeneJuice™: personalized, custom-blended dispensed beverages, adjusted for an individual's genetic chemistry, to enhance physical and mental ability. Delivered through specially designed vending machines that will mix the drink on-the-spot based upon the person's genetic profile and the current activity. The formulation process uses the results of a one-time scientific analysis of a person's cheek cells, from a kit that can be used at home. Other related personalized nutraceutical products: Health For You™: a customized vitamin-mineral supplement base on biochemical profiling Pink Mammoo™: a natural breast health product Ace of Hearts™: a natural ACE inhibitor SkyGene™: provides a personalized view of a person's many normal genes that may be important in developing social relationships. The basic package is a hand held microelectronic device with ability to store and process data, and to display the person's genetic profile as a holographic image.
Genelex	www.genelex.com	Provides DNA testing services to medical professionals and the general public. Personalized nutritional and lifestyle recommendations based on genetic testing. Testing examines 19* genes related to heart and bone health, B vitamin use, detoxification, antioxidants, inflammation and insulin sensitivity.	Genetic testing combined with a lifestyle assessment Nutritional Genetic Panel DNA Diet Rapid Results DNA Diet Weight Loss System These are available with or without nutritionist consultation and menu for health optimisation or weight loss. Price: \$395-1200. The DNA sample (cheek cells) is taken at home in a sample collection kit and mailed together with a completed questionnaire (eating and other habits).
HortResearch Auckland, New Zealand	http://www.hortresearch.co.nz	A fruit science company, uses the unique resources in fruit, plant and environmentally sustainable production systems to produce innovative fruit and food products. A partner in Nutrigenomics New Zealand (a centre of excellence).	Advices companies on how nutrigenomics might impact on their business. Assists companies to develop their 'nutrigenomic' foods with proven efficiency against specific conditions.

<p>Interleukin Genetics Waltham, MA, USA</p>	<p>http://www.ilgenetics.com</p>	<p>Is among the world leaders in understanding and applying the genetics of inflammation, one of the body's most important defense mechanisms, to improve health. Develops genetic tests to inform people about an increased risk for disease, and preventive and therapeutic products to reduce the risk of those diseases.</p> <p>The goal in the nutrigenomics sector is to partner with leaders in the food and nutrition industry to develop nutritional products that extend wellness by first identifying those individuals with specific gene variations and then to provide products to those persons. These targeted products will be in the form of specially configured nutritional supplements or functional foods.</p>	<p>Gensona™ Heart Health Genetic Test: IL1 gene test to identify an individual's predisposition for over-expression of inflammation and increased risk for CVD and acute cardiac events. Price: \$199</p> <p>Gensona™ General Nutrition Genetic Test: identifies nutrient metabolism, including vitamin B (MTHFR, TCN2) and antioxidants (SOD2, GSTM1, PON1, XRCC1). Price: \$99.95</p> <p>NUTRILITE IL1 Heart Health Nutrigenomic Dietary Supplement (90 tablets, \$69.95): recommended for individuals tested positive for an over-expression of the IL1 gene. Contains rosehips, grape stem and vine extracts, blackberries and blueberries (antioxidants).</p>
<p>Metagenics San Clemente, CA, USA</p>	<p>http://www.metagenics.com</p>	<p>“To improve health by helping people achieve their genetic potential through nutrition”. Focuses on the application of targeted, personal nutrition to help foster positive genetic expression and healthy eating.</p> <p>Develops and manufactures natural, science-based nutraceuticals. Employs advanced new technology to produce formulations that manifest ‘multifunctional synergy’ – the ability to support health genomics, proteomics and metabolomics.</p> <p>ExpresSyn Process has been developed for demonstrating the efficacy, bioavailability and safety for the new line products, combining cell proteomic research, safety evaluations, human ex vivo research and human clinical research.</p>	<p>Several lines of nutraceuticals, which are only available through licensed healthcare practitioners. An example:</p> <p>Metabolic Detoxification for Imbalanced Detoxifiers UltraClear PLUS® & UltraClear® Plus pH Medical Food</p> <p>pH personalized formulas for all 3 phases of detoxification</p> <p>UltraClear PLUS is designed for those with imbalanced detoxification capacity, or low Phase II activity, who exhibit a urinary pH above 6.6. Supplies vitamins, minerals, and accessory nutrients to support both Phase I and Phase II detoxification (low-allergy-potential rice protein concentrate with added limiting essential amino acids L-lysine and L-threonine, rich in the antioxidant vitamins A, C, E, and beta-carotene) and glycine, taurine, sodium sulfate, and catechins from decaffeinated green tea to better support Phase II activities.</p>
<p>NutraGenomics Chicago, IL, USA</p>	<p>http://www.nutrigenomics.com</p>	<p>A research and development biotechnology company dedicated to improving human health by providing nutritional solutions to chronic and stage-of-life diseases. Develops novel diagnostic tests and identifies new, targeted therapies</p>	<p>Contract research activities:</p> <ul style="list-style-type: none"> - Gene expression profiling - Analyses of nutraceuticals, pharmaceuticals, and nutrients using expression profiling - SNP analyses - Feeding regimens in inbred mouse strains

		for diseases like type 2 diabetes, obesity, atherosclerosis, and certain cancers. Using systems biology approach, it will identify diet-regulated genes and nutritional interventions that will allow individuals to better manage their health and well-being (intelligent nutrition)	<ul style="list-style-type: none"> - Highly controlled environmental conditions - Data analyses and storage
Salugen San Diego, California, USA	http://www.salugen.com	<p>“DNA Customized Nutrition” – targeting structural, functional and systemic deficiencies with genetically guided nutritional solutions. Provides DNA-customized nutritional products to address specific imbalances revealed through genetic testing.</p> <p>A simple DNA test is used to determine genetic imbalances, and then a customized blend of nutritional elements is formulated to correct those imbalances.</p>	<p>GenoScore™ genetic test: a series of genes that indicate specific mutations that may cause nutritional imbalances (cheek cells). Price: \$399.95</p> <p>GenoTrim™: a DNA-customized nutritional supplement for weight, works directly on the specific genetic pathways that may contribute to tendencies to gain and retain weight. Price: \$209.95</p> <p>Targets the following genes:</p> <ul style="list-style-type: none"> - Obesity Risk Gene™ - leptin gene - Nervous Eating Gene™ - 5-HT-2a receptor SNP - 1438G/A - Sweet Tooth Gene™ - Dopamine D2 receptor gene - New Cell Gene™ - methylene tetrahydrofolate reductase gene mutations C677T and A1298C - Fat Regulator Gene™ - PPARγ gene <p>Some of the primary ingredients include: Garcinia cambogia extract, niacin-bound chromium and folic acid.</p>
Sciona Boulder, Colorado, USA	http://www.sciona.com	<p>“Optimal health through genetics”</p> <p>Provides personalized health and nutrition recommendations based on an individual’s diet, lifestyle and unique genetic profile. Utilizes genetics to aid companies in developing more effective, individually formulated products that may help consumers live healthier lives. Also sells nutrigenetic assessment kits directly to consumers through multilevel marketers, catalogs and via the Internet.</p>	<p>Cellf™ Genetic Assessment: a DNA, diet and lifestyle assessment analysing a total of up to 19* genes and gene variations. These genes and gene variations are then grouped into each of five important health areas - heart health, bone health, insulin resistance, antioxidant/detoxification, and inflammation. Prices: \$126, comprehensive (all 19 genes) \$252. A personalized report is generated with an action plan comprised of specific diet, nutrition and lifestyle recommendations tailored to the unique genetic profile.</p>
WellGen New Brunswick, NJ, USA	http://www.wellgen.com	<p>”Wellness Generation”</p> <p>This biotechnology company, a spin-off from Rutgers University, develops health and wellness products using gene expression platform technology. Uses its patented screening technology to screen naturally occurring compounds that are</p>	<p>The first products: anti-arthritis compound and anti-colon cancer compound</p> <p>Next products: compounds in the areas of anti-obesity, exercise recovery and exercise performance.</p>

		generally regarded as safe, against a panel of genes associated with wellness and disease. Has identified several compounds found in food that alter expression of genes associated with cancer and inflammation (e.g. an ingredient derived from orange peel and theaflavins from black tea). Products based on these substances are being developed and will be marketed as dietary supplements, functional foods and cosmeceuticals.	
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* The 19 genes tested:

MTHFR - 2 variations

MS-MTRR

MTR

CBS

MnSOD - 2 variations

SOD3

GSTM1

GSTT1

GSTP1 - 2 variations

IL-6 - 2 variations

APOC3

CETP

LPL

eNOS

ACE

VDR - 3 variations

COL1A1

TNF- α

PPAR γ

APPENDIX 4. EU Fifth and Sixth Framework Programme projects with elements of nutrigenomics

Project	Coordinator	Web site	Status	Nutrigenomics element
<i>Fifth Framework Programme</i>				
NUTRIGENOMICS A European workshop on molecular approaches to human nutrition research	I. Lester, Institute of Food Research, Norwich, UK	-	Completed 1.5.-31.10.2001	This workshop examined the role of diet-gene interactions in chronic/degenerative diseases. It brought together established scientists/junior researchers from the areas of bioinformatics, functional genomics, transcriptomics, proteomics, nutrition and food science. It stimulated communications between researchers, defined the challenges, opportunities and priorities in the area, identified key knowledge gaps and established a basis for effective inter-disciplinary and academic/industry exchange.
NUGENOB Nutrient-gene interactions in human obesity: implications for dietary guidelines	T.I.A. Sorensen, Institute of Preventive Medicine, Copenhagen University Hospital, Denmark	http://www.nugenob.org/	Completed 1.3.2001- 29.2.2004	The aim was to improve understanding of the interaction between nutrition, especially fat intake, and genetic variations in obesity. Obese subjects from eight European cities underwent a 10-week hypocaloric dietary intervention with either high- or low-fat content, and changes in adipose tissue gene expression were assessed. Novel nutrient-sensitive and candidate genes and gene variants were identified.
DIET AND OBESITY Dietary and genetic influences on susceptibility or resistance to weight gain on a high fat diet	J.G. Mercer, Rowett Research Institute, Aberdeen, UK	http://www.eurobesity.org http://www.adipositas-online.com	Completed 1.2.2001- 30.6.2004	The aim was to describe phenotypes associated with dietary preference and relative susceptibility or resistance to weight gain and obesity on a high-fat diet, and investigated the genetic basis of this preference and susceptibility in human volunteers and patients.
OSTEODIET Optimal nutrition towards osteoporosis prevention: impact of diet and gene-nutrient interactions on calcium and bone metabolism	K. Cashman, University College Cork, National University of Cork, Cork, Ireland	http://osteodiet.ucc.ie/	Completed 1.2.2000- 31.10.2003	The aim was to investigate the influence of diet (Na, Ca, protein, vitamin E) and its interaction with individual genetic variation (genes for vitamin D receptor, apolipoprotein E) on the metabolism of calcium and bone in order to reinforce the scientific base for dietary strategies for osteoporosis prevention in Europe.
FFAC Functional food ingredients against colorectal cancer - development of a genomics and proteomics based screening assay	R.A. Woutersen, TNO Nutrition and Food Research, Zeist, NL	-	Completed 1.1.2000- 31.12.2002	The aim was to develop an in vitro bioassay, based on genomic and proteomic changes in colorectal cells, to assess the efficacy of (functional) food ingredients in prevention or inhibition of the development of early stages of colorectal cancer.

Project	Coordinator	Web site	Status	Nutrigenomics element
<i>Sixth Framework Programme</i>				
NUGO The European Nutrigenomics Organisation - linking genomics, nutrition and health research	B. van Ommen, TNO Nutrition and Food Research, NL	http://www.nugo.org	Ongoing 2004-2009	The network (23 participants from 10 countries) is creating a virtual centre of excellence to integrate and develop genomic technologies for the benefit of European nutritional science, to facilitate the application of these technologies in nutritional research and train a new generation of scientists to use them. The network does not do research as such. Its purpose is to overcome the fragmentation of research, giving researchers from across Europe the chance to work together, share facilities and their expertise. The main deliverable is a durable structure and clear strategy for future research.
LIPGENE Diet, genomics and the metabolic syndrome: An integrated nutrition, agro-food, social and economic analysis	M. Gibney and H. Roche, Institute of Molecular Medicine and Department of Clinical Medicine, Trinity College, Dublin, Ireland	http://www.lipgene.tcd.ie/	Ongoing 2004-2009	The focus of this project is the interaction between dietary fat composition and genotype in the metabolic syndrome. Access to a large prospective cohort, together with clinical and nutritional data, provides a unique opportunity to determine diet-gene interaction in the development of the metabolic syndrome. A large dietary multi-centre intervention study will examine how dietary fat modifications, varying in both fat content and composition, influence insulin sensitivity and other aspects of the metabolic syndrome, taking genetic differences into account.
DIOGENES Diet, Obesity and Genes	W.H.M. Saris, Nutrition and Toxicology Research Institute Maastricht NUTRIM, University of Maastricht, NL	http://www.diogenes-eu.org/	Ongoing 2005-2009	The project will focus on gene-nutrient interactions associated with changes in body weight. It will identify and characterise biomarkers of dietary intake with variations in carbohydrate glycaemic index (GI) and protein content and predictors of weight and metabolic variations. Three approaches will be used: <ul style="list-style-type: none"> • genetics, addressing how variants in the coding and the regulatory regions of candidate genes affect overall weight regulation, and whether certain genotypes are associated with a differential response to different dietary GI levels or protein content. • transcriptomics, addressing whether gene expression in adipose tissue predicts weight loss and weight gain patterns, and how gene expression is altered in response to changing the dietary protein content or GI. • proteomics/peptidomics, addressing whether proteins or peptides in the blood predict weight loss and weight gain patterns, whether some peptides predict a differential response to different diet compositions, and finally how protein and peptide levels alter in response to changing the dietary protein content or GI.

Project	Coordinator	Web site	Status	Nutrigenomics element
NUCSYS Systems biology of nuclear receptors: A nutrigenomics approach to aging-related diseases	C. Carlberg, Department of Biochemistry, University of Kuopio, Finland	http://www.uku.fi/nucsys/	2006-2009	The co-ordinated activities of the nuclear receptor (NR) transcription factor family orchestrate responses to environmental factors by sensing a diverse range of dietary and xenobiotic components. The main goal of this project is to define key components of the NR gene regulatory network that facilitate this process and model their impact on the development of aging-related syndromes and pathologies, such as carcinogenesis, metabolic syndromes, osteoporosis and skin aging. The aims are: I. to use interdisciplinary expertise in applying standardized protocols to study the impact of dietary sensing by NRs on the genome, epigenome, transcriptome, proteome, metabolome and physiome, and thereby model these actions in different human tissues. II. to use intersectorial expertise in revealing the significance of NR-nutrient signaling in pathological conditions associated with aging. III. to generate an interdisciplinary and intersectorial understanding using systems biology modelling with the aims of i) revealing a predicative and preventative ultimately personalized paradigm for the effect of diet upon key aspects of health within the aging European population and ii) generating commercial realization.
ZINCAGE Nutritional zinc, oxidative stress and immunosenescence: biochemical, genetic and lifestyle implications for healthy ageing	E. Mocchegiani, Immunology Center, INRCA, Ancona, Italy	http://www.zincage.org/	2004-2007	The project will study the role of zinc in the gene expression and functions of metallothioneins, chaperones, NO, MsrA, ApoJ, telomerase, PARP-1 and DNA-repair as a function of immunosenescence in lymphocytes from old subjects including nonagenarians, as well as in ill old patients affected by age-related diseases and in T-cell clones as an in vitro model. Particular attention will be paid to the processing of oxidised proteins by proteasome, which decreases in ageing. The activity of all proteins herein studied is impaired in ageing and this is directly and indirectly controlled by zinc ion bioavailability. The zinc deficiency in the elderly will be evaluated through the screening of polymorphic allele frequency of proteins involved in zinc ion metabolism (such as Hsp70-2 and TNF) and their relationships to gender. The results will form a rationale for the promotion of healthy ageing through a zinc supplementation.