Application of new technologies and methods in nutrition research – the example of phenotypic flexibility

**Reporting**

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**RESULTS PACK**

Innovation and exploration through cutting-edge Microbiome research

16 October 2017
Executive Summary:
The EC funded FP7 project NutriTech demonstrated the combined use of all major nutrigenomics technologies in a human intervention study to detect all relevant changes after a subtle dietary restriction. The goal of this study thus was two-fold: assess the use of the technologies, and identify new biomarkers that can be used in conditions very close to optimal metabolic health. This second goal was based on the concept of “phenotypic flexibility”. Optimal metabolic health depends on the proper functioning of a complex system of metabolic processes that continuously switch from anabolic to catabolic conditions, is guided by a fine-tuned set of regulatory processes and involved many organs (primarily intestines, pancreas, liver, muscle and adipose). A set of simplified biomarkers (body weight, BMI, fasting plasma glucose, free fatty acids, triglycerides, cholesterol) are commonly used in nutrition and health studies. NutriTech exploits phenotypic flexibility and in-depth quantifies metabolic response reactions upon standardized metabolic challenges. On top of the standard parameters, the technologies used were transcriptomics of PBMC, lipidomics and metabolomics of plasma, NMR-profiling of lipoproteins, targeted plasma proteomics, all during the time course of metabolic tolerance tests (glucose and mixed meal, also including a physical exercise challenge. These assays were complemented with whole body MRI, and transcriptomics, proteomics of muscle and adipose tissue, intestinal microbiome sequencing, DNA integrity analysis and whole exome DNA sequencing. The biomarker outcomes of the intervention study were compared with biomarker profiles of identical or similar challenge tests in observational cohorts or 3-year dietary intervention studies, thus relating the observations in healthy conditions to aging or disease development. Also, the NutriTech biomarkers results were evaluated together with a number of other studies focusing on aspects of phenotypic flexibility.

Parallel to the phenotypic flexibility research, the same metabolomics technologies were applied in the area of food intake quantification. Standardized meals with varying percentages and origins of protein were provided to human volunteers, and plasma and urine metabolomics identified food constituent specific quantitative biomarker (profiles). The markers were confirmed in cohorts and open access databases were constructed. This effort indicates a new manner of quantification of food intake.

A few remarks need to be made. The NutriTech intervention study subjects were probably, at the time of execution, the most extensively quantified subjects. The study created an enormous dataset. The analysis created a challenge and at the time of formal closure of the project, still has many avenues to explore. Open collaborations and connected open data access are logical next steps. Also, the detailed geno-and phenotyping revealed a number of subgroup-specific effects, and even unique individual genotype-phenotype combination results on top of the originally intended average intervention. This suggests that alternative data analysis strategies should be explored, valorising the inter-individual differences in response.

NutriTech closely collaborated with major food industry partners in a “twinning project”. Regular joint meetings, data exchange and four symposia (two in Europe, one in South Korea and one in the USA) contributed to a strong dissemination of the concept of phenotypic flexibility as biomarker in nutrition and health research, and the results of NutriTech.
Project Context and Objectives:
NutriTech has built on the foundations of traditional human nutrition research using cutting-edge analytical technologies and methods to comprehensively evaluate the diet-health relationship and critically assess their usefulness for the future of nutrition research and human well-being. Technologies included genomics, transcriptomics, proteomics, metabolomics, laser scanning cytometry, NMR based lipoprotein profiling and advanced imaging by MRI/MRS.

All methods were applied in an integrated manner to quantify the effect of diet on “phenotypic flexibility”, based on metabolic flexibility (the capacity for the organism to adapt fuel oxidation to fuel availability) but extending the area of flexibility to all processes and mechanisms involved in absorbing metabolic challenges, which are essential in maintaining optimal metabolic and inflammatory health. We have coined this extended view on flexibility “phenotypic flexibility”. Biomarkers reporting on processes related to health thus report on the mechanisms that retain optimal stress responses after a metabolic / caloric challenge. So, NutriTech has moved beyond the state-of-the-art by applying the above mentioned and integrated technologies to assess the underlying and related cell biological and genetic mechanisms and multiple physiological processes of adaptation when homeostasis is challenged. Methods were evaluated within a human intervention study, which has delivered a package of biomarkers that fully exploit the power of new technologies, and that focus on quantifying all relevant aspects of phenotypic flexibility. The resulting optimal methods and biomarkers panels were validated in a number of existing cohorts against established endpoints.

NutriTech has disseminated the harmonised and integrated technologies on a global scale by a large academic network including 6 non-EU partners and by providing an integrated and standardised data storage and evaluation platform. The project has generated a unique dataset on extensively phenotyped and genotyped subjects in the context of a dietary intervention study, revealing a multitude of minor changes. The impact of NutriTech is multi-fold and exploitation is crucial as major breakthroughs from our technology and research are expected. The collaboration with a consortium of five global food industries and the exploitation of specific technologies by our five SME partners contributed to the project’s impact. Overall, NutriTech has built the foundations for successful integration of emerging technologies into nutrition research, and has produced a new generation of biomarkers of health.

The strategic goals of NutriTech were:
1. Evaluate the value of emerging technologies in the quantification of subtle effects of dietary interventions on health;
2. Evaluate the added value of emerging technologies to elucidate mechanisms of action in human studies;
3. Validate the use of emerging technologies for studying human metabolic and physiological adaptive processes in response to a shift from a suboptimal to a healthy diet;
4. Develop the integrated quantification of aspects of phenotypic flexibility as biomarkers of diet-related health improvement;
5. Develop methods integrating established as well as emerging technologies to study nutritional effects on health;
6. Provide guidelines and protocols to harmonise the use of the developed integrated methods;
7. Establish a data infrastructure in a global network of laboratories to disseminate and implement the new methods and technologies developed in nutrition research;
8. Valorise the new integrated technology for the food industry in a renewed effort to demonstrate health benefits.
benefits of defined dietary interventions.

Project Results:
The NutriTech project started in January 2012 and ran until June 2016. The consortium has disseminated the results and outcomes through presentations, workshops, scientific articles, print media and a video animation. NutriTech has built the foundations for successful integration of emerging technologies into nutrition research, and has produced a new generation of biomarkers of health, through the activities in its work packages.

One of the new technologies implemented related to food intake metabolomics, which was the topic of WP1. Overall, this WP has made significant contributions to the field of dietary biomarkers. It has laid the foundations for the development of concepts such as nutritypes, use of panel of biomarkers for dietary intake assessment and integration of biomarker and dietary data. More details on these topics are included in the following paragraphs.

Food Metabolite Databases play a key role in the development of the area of Food Intake Metabolomics. WP1 contributed to the development of The Food Database, FooDB through the University of Alberta. This is a database of food constituents for raw or processed foods and was built to collect information on all known food compounds. The corresponding data (>13,000 compounds) has been integrated in the Human Metabolome Database. Both databases are fully accessible to the scientific community at [http://foodb.ca/](http://foodb.ca/) and [http://www.hmdb.ca/](http://www.hmdb.ca/).

Food intake markers were identified for red meat, chicken and fish consumption using data from the NutriTech food intake study which was performed as part of WP2. All meals within the NutriTech food intake study were designed to provide similar intakes of dietary energy and fibre but the quantity of foods changed from week 1 to week 3; for example red meat increased from 81g to 288g in females and 95g to 340g in males from week 1 to week 3. Through a combination of metabolomics and multivariate data analyses procedures, a series of markers specific for the food sources were identified. The markers were then confirmed either in the EPIC cohort or the NANS cohort. Urinary nitrogen was confirmed as a robust biomarker of protein intake across a range of different protein sources. Furthermore, potential markers reflective of dietary fat intake were identified using a cohort (MECHE: metabolic challenge study) and confirmed as being responsive to increasing fat intake using the NutriTech food intake study.

Measurement of biomarkers related to food intake is of interest to the nutrition community and development of a panel of such markers that can be measured together as a set would represent a move towards making these markers available to the wider community. To this end a feasibility study for the nutrition-specific extension of Biocrates’ AbsoluteIDQ p180 quantification assay for metabolites related to food intake was performed. The assay was extended to included 8 additional metabolites (1-methylhistidine, 3-methylhistidine, TMAO, para-hydroxy-phenyl-acetic acid, dimethylamine, anserine, DHA, EPA) resulting in a “Nutritional assay”. The technical feasibility study was successful for 6 metabolites: 1-methylhistidine, 3-methylhistidine, TMAO, Anserine, DHA, EPA. The newly established “Nutritional assay” was applied to a human sample cohort, comprising 120 plasma samples from healthy subjects prior to and following consumption of red meat. The data evaluation revealed that the assay reliably measured the biomarkers and that the intake of red meat resulted in strong concentration changes in several metabolites, especially, as expected, TMAO, 1-methylhistidine, 3-methylhistidine. The results indicate that the concept is feasible and could be developed to include further markers.

The concept of defining dietary patterns using a marker profile (nutritypes) was examined and developed
Using an Irish Cohort we explored the possibility of defining nutritypes and examined their potential use. Two nutritypes were identified using the metabolomic profile data: Cluster 1 was described as the “healthy dietary pattern” cluster due to the higher mean percentage intake of the more nutritionally favourable food groups and cluster 2 could be described as the “unhealthy dietary pattern” cluster due to the higher mean percentage intake of the more energy dense and nutritionally undesirable food groups. Nutrient and biochemical profiles also highlighted significant differences between the clusters that were reflective of their food group intake. Participants from the NutriTech food intake study (n=39) were used to investigate the ability of this model to classify people into different dietary patterns. Use of this model revealed that 95% of subjects were placed into the correct dietary pattern (according to the information we had on their dietary intake).

Integration of dietary data and metabolite measurements was also explored within this WP. An algorithm to integrate a panel of urinary polyphenol metabolites with self-reported dietary assessments (24-hour dietary recall and food frequency questionnaire measurements) for the identification of relevant biomarkers of dietary intake was developed. An analytical framework to relate profiles/patterns of 34 urinary polyphenols to predict intake of polyphenol-rich foods, applying a multivariate statistical technique, reduced rank regression (RRR) was developed.

The NutriTech consortium conducted an extensive dietary intervention study to explore phenotypic flexibility by applying a wide range of emerging technology. The study was coordinated by WP2 and had the following objectives:

1. To provide an environment to carry out the metabolomic food intake biomarker enquiry.
2. Investigate the use of metabolomics profiling as a method of independent food quantification
3. To quantify the effect of diet on ‘phenotypic flexibility’ (adaptation of biological and physiological processes in the state of challenged homeostasis).
4. To combine classical approaches of human nutrition research with new analytical technologies and methodologies (whole genome genetics and epigenetics, transcriptomics, targeted proteomics, metabolomics and lipidomics, magnetic resonance imaging, laser scanning cytometry) to assess their usefulness in nutrition research.

The intervention study was designed in to parts, firstly volunteers completed metabolomics biomarker study (WP1), following which the volunteers undertook trial to explore the impact of energy restriction on phenotypic flexibility. The study received an ethical approval from Brent Ethics Committee (REC ref: 12/LO/0139). Volunteers were recruited from London area between June 2012 and April 2014. To minimise variability in the study we decided to run one major highly controlled trial on one site. Here we will focus on the nutritional intervention for exploring phenotypic flexibility.

We undertook a randomised control trial where volunteers where randomised to:

1. Intervention diet aimed at reducing energy intake by 20% with the diet complying to healthy eating
2. Control diet aimed at weight stability and will have a macronutrient profile of 45% of total energy from carbohydrate, 40% from fat and 15% from protein.

The dietary intervention lasted 12 weeks and was completed by 72 subjects (35 males and 37 females) over a period of nearly 2 years. At the beginning and end of the 12 week period volunteers undertook a 4 day period on investigation.

Day 1 Anthropometrics (height, weight, waist circumference), Food Frequency Questionnaire and 7 day dietary diary, Seven-Day Physical Activity Recall (PAR) Questionnaire, Indirect calorimetry for respiratory quotient and energy expenditure, imaging analysis by MRI, collection of peripheral blood mononuclear
cells (PBMC), collection of buccal mucosa cells, fasting blood sample.

Day 2: Frequently sampled oral glucose tolerance test (OGTT), to detect change in insulin sensitivity and beta cell function.

Day 3: Standard Liquid Diet challenge with 6h (6 time point) plasma sampling, with adipose tissue and muscle biopsies prior and after 6 h time course. This was be used to assess post prandial nutrient handling including post prandial insulin sensitivity.

Day 4: Standard Liquid Diet challenge combined with a physical exercise test, with 6h (6 time point) plasma sampling, with adipose tissue and muscle biopsies prior and after 6 h time course. This allows for assessment how exercise effects the phenotypic flexibility.

We contacted 22,000 people by post, from which 2900 expressed interest in taking part, screening over the telephone reduced this number to 210 of whom 191 were screened for the study exploring phenotypic flexibility. 81 people were randomised of which 72 completed the study (32 in the control group and 40 in the intervention group).

Summary of results:

Our intended weight loss target was successfully achieved with a weight loss of -5.4 ± 2.5 kg in the interventional group, and minimal weight change -0.23 ± 2.3 kg in the control group. Although there was no difference of the effects of the intervention on glucose and insulin profiles, there was a significant improvement in insulin sensitivity as assessed by HOMA IR and HBA1c. There was a weak but significant correlation between changes in body weight and changes in both fasting glucose (r=0.37 p=0.0011) insulin (r=0.27 p=0.014) and HOMA%S (r=−0.34 p=0.003). Significant reductions were observed in total and regional adipose tissue content as well as ectopic fat content in the liver and soleus muscles (see body composition analysis in WP4). These phenotypic changes were accompanied by improved in fasting insulin sensitivity and a reduction in HbA1c in the weight maintenance group.

Blood samples were distributed to different NutriTech partners for analysis (WP3, WP5, WP6). In the end, the consortium has generated a unique dataset on extensively phenotyped and genotyped subjects in the context of a dietary intervention study, revealing a multitude of minor changes.

WP3 focused on measurement and interpretation of systemic biomarkers of phenotypic flexibility. Human metabolism has to adapt in response to food intake or to fasting with the use of different energy substrates and initiated by changes in hormone levels. In this respect human metabolism changes constantly in a biochemical and physiological sense between a catabolic (i.e. overnight fasting) and anabolic states (after food intake) and the adaptation to respond in time and space (i.e. organs) to these challenges in an individual is defined as “phenotypic flexibility”.

WP3 was dedicated to assess at the systemic level, mainly based on blood samples (and partly also urine and biopsy samples) whether volunteers can be classified by their capability to respond and whether the intervention can improve the responsiveness. It is generally well accepted that the flexibility becomes significantly impaired when individuals move from a healthy to a prediabetic or diabetic state. This is best visible by the major differences in the capability to control the changes in blood levels of glucose following a defined load of carbohydrates or test meal. Within NutriTech this capability was tested by submitting the volunteers to an oral glucose tolerance and a mixed meal test (including one with some exercise build in) and by repeating the same tests after 3 months in volunteers subjected or not to 20% energy restriction. Analysis of the biosamples was in WP3 based on multiple analytical platforms determining “classical” clinical-biochemical biomarkers (from cholesterol to markers of inflammation and stress response to reactive oxygen species) as well as a large number of
hormones, cytokines and hundreds of metabolites. Within the metabolite spectrum, analysis platforms such as nuclear magnetic resonance (NMR) or mass-spectrometry methods based on gas-chromatography or liquid-chromatography delivered a wide spectrum of lipids, amino and other organic acids, sugars and wide spectrum of other intermediates. On basis of this comprehensive analysis – almost unique in the field of human nutrition research – the concept of phenotypic flexibility was tested for its validity.

Although no major effect of the weight loss was found on the concentration of almost all entities measured in blood by WP3, it became obvious that the huge variability observed in the volunteer’s response to the challenge tests is the real asset of the study. It is indeed remarkable to see how well and to which extent the volunteers reproduced their own time-profiles for hundreds of metabolites and many hormones when undergoing the same tests at the beginning of the study and after 3 month for follow up.

We defined a so called “identity index” as the difference in the plasma levels over time for hundreds of metabolites and hormones to the glucose tolerance or the mixed meal tests before or after the 3 month period. This identified around 70 entities that displayed an > 80% identity in the time-profiles. This means, inter-individual differences in the responses to the same challenge are remarkable. Yet, these responses are extremely reproducible in an individual over at least 3 month suggesting very strong genetic and physiological determinants even of the dynamics of changes. NutriTech will eventually be able to deliver answers on what determines these major phenotypic differences by assessing on how genetic variables (based on whole exome sequencing data collected) but also on microbiome information obtained from fecal samples.

There is also good evidence that the volunteers in NutriTech can be separated into two clusters based on the plasma profiles of metabolites that derive from metabolic processes. These two clusters comprise very similar number of volunteers and also in numbers on men and woman. This separation of volunteers into two clusters is based on differential kinetic response of markers of lipid catabolism in plasma, induced by glucose and insulin after meal/glucose intake. Most importantly, only one of the two clusters showed significant improvements in the parameters when analyzed after the weight loss. Individuals that separated into the cluster with impaired responses were found to have significantly higher liver fat content and displayed also higher plasma gamma-glutamyl transpeptidase activity, among other markers of insulin resistance, diabetes and hepatic function.

Taken together, WP3 has in the framework of NutriTech delivered an impressive portfolio of analytes that for the systemic level identified new biomarkers from physiological processes that indeed demonstrate impaired phenotypic flexibility in humans based on how they cope with a dietary challenge. Moreover, this comprehensive phenotyping approaches defined “metabotypes” as subgroups with a benefit from the intervention that induced the predicted weight loss, despite the fact that no major improvements at the whole study group level were observed. The further analysis is described in Workpackage 7, which deals with the integration of all results.

As rates of obesity and associated disease escalate globally, the role of body fat content and distribution has elicited much interest and it is recognised as a key indicator of risk. Thus, the main focus of WP4-Imaging) was to quantify, in an accurate and objective manner, changes in fat content and distribution in the NutriTech human intervention study and develop potential alternatives to the current “gold-standard” for body composition, principally magnetic resonance imaging (MRI).

Following the intervention, there were significant reductions in both total and regional adipose tissue content (p<0.001). The mean total fat loss was -13.0 ± 6.1%. The magnitude of the reduction was body fat
depot specific, with the greatest change being observed for the visceral fat (-18.5 ± 13.0%), followed by the subcutaneous fat (-15.3 ±7.9%). The smallest reductions were observed in the non-abdominal fat (-10.4 ± 12.3%). This suggest a preferential reduction from abdominal compared with peripheral regions of the body.

Ectopic fat depots also showed significant reduction, with liver fat content being the largest (-47.8%; p=0.002). There was a similar magnitude reduction in pancreatic fat content (-24.7%; p=0.06). Similarly, fat levels were significantly reduced in the soleus muscle (-3.2 ± 5.3; p=0.012) but not in the tibialis muscle.

When these results were analysed with respect to gender it was found that while there were no significant gender differences in total or subcutaneous adipose tissue lost in response to the weight loss intervention, there were gender differences in the amount of internal adipose tissue lost. Male subjects lost significantly more total internal (p=0.01) and visceral (p=0.001) fat compared with female volunteers. There was no impact of gender on the amount of ectopic fat lost following the weight loss intervention.

Change in body weight and fat mass and distribution were accompanied by significant reductions in both waist (p<0.001) and hip circumferences (p<0.001). Again there was a very strong gender bias in the pattern of body-shape changes, with male subjects showing significantly large changes in waist, while females showed a larger change in hip circumference.

These results show the power of new technology in assessing the impact of lifestyle intervention on body composition. However, this methodology is expensive and time consuming so alternatives, based on anthropometric and metabolic variables were investigated. Significant correlation between different fat depots and these variable were observed, however their predictive values were rather limited. This partially reflects the large biochemical variability of humans, together with the relatively limited size of the NutriTech cohort. Attempts to validate some of these finding in larger cohort were not fruitful as they either lacked the depth of metabolic profiling undertaken as part of the NutriTech study or did not have in-depth body composition analysis.

An interesting outcome of these modelling was the novel discovery of the psoas muscle as a potential biomarker for fitness and muscle function. The psoas major is a large muscle of the abdomen, forming part of what is commonly referred to as the ‘core muscle group’. We observed a significant correlation between psoas muscle size and VO2max (P < 0.0001). This was not observed with other muscle groups investigated in NutriTech, once weight, BMI and age were taken into account. The association between psoas muscle and fitness was independent of gender.

In conclusion, circulating metabolome were found to be poor predictors of visceral and ectopic fat levels in this cohort. However, they appear to be good predictors of weight loss, while psoas muscle appears to be a strong predictor a person physical fitness, which may be an invaluable tool to the study of frailty and old age sarcopenia.

WP5 studied phenotypic flexibility of organs, specifically peripheral blood mononuclear cells and skeletal muscle and adipose tissue. An important technology used in this workpackage was transcriptome analysis.

Unfortunately, the NutriTech study resulted in limited intact pairs of muscle and adipose tissue biopsies. The samples available were used for histological analysis (muscle biopsies) and transcriptome and metabolome analysis (adipose tissue). For studying phenotypic flexibility of muscle, we used a comparable study (MyoGlu study).

We fixated muscle and adipose tissue biopsies before embedding and immunohistochemistry. Muscle
samples from the NutriTech intervention study we only available to a limited extent: 3 intact sample pairs have been embedded in paraffin, both from Day1 (D1) and day 4 (D4). This methodology has been validated on biopsies. From a comparable study described above (the Myoglue study), we embedded 36 muscle biopsies for electron microscopy (EM) histology. Another 14 muscle biopsies are prepared for glycogen enhanced EM analysis, in addition to 36 adipose tissue biopsies embedded for EM histology. Recently, we published the largest and most thorough EM analysis of lipids in human muscle ever conducted (> 2800 EM images analyzed by a database assisted approach), finding that subsarcolemmal lipid droplets are strongly affected (~80% decrease) by exercise (Li et al., 2014).

Muscle results:
To explore metabolic flexibility the effects of in vivo exercise on in vitro metabolic adaptations, we studied energy metabolism, insulin signaling, and gene expression in cultured myotubes established from biopsies from healthy sedentary men with normal weight and overweight. Biopsies were obtained from the “MyoGlu” study, before and after 12 weeks of physical training, including endurance as well as strength exercise sessions, each lasting 60 min with carefully supervised activity. All together 11 overweight and 7 normal weight voluntary men were included in the study based on the fact that they should have been physically active not more than once a week the previous year.

The training intervention improved endurance, strength, and insulin sensitivity in vivo. Biopsy-derived cultured human myotubes from overweight subjects after training intervention showed increased oleic acid uptake (30%), oxidation (46%), lipid accumulation (34%), and fractional glucose oxidation (14%) as compared to cultures established prior to exercise. Myotubes established after exercise confirmed these results on mRNA expression level. Based on these data we conclude that 12 weeks of combined endurance and strength training promoted increased lipid and glucose metabolism in biopsy-derived cultured human myotubes, showing that effects of training in vivo are conserved in human myotubes in vitro.

Interestingly, in our studies we also demonstrated that previously published work by others in Nature, reporting on a specific muscle-derived signalling protein (“myokine”) called Irisin was incorrect, due to non-specificity of their antibody-based detection system.

Overweight and obesity lead to changes in adipose tissue such as inflammation and reduced insulin sensitivity. We compared the NutriTech study subjects (~20% reduced intake of energy from food), to the MyoGlu study (enhanced energy expenditure due to physical exercise). We monitored mRNA expression, by microarray and RNA sequencing, from adipose tissue biopsies. The comparison of microarray and RNA sequencing showed excellent correlations, which were also confirmed with RT-PCR. In the NutriTech energy restricted subjects there were clear signs of enhanced lipolysis as monitored by mRNA in adipose tissue as well as plasma concentration of free fatty acids. This increase was strongly related to increased expression of markers for M1-like macrophages in adipose tissue. In the exercising subjects (glucose infusion rate increased by 29% during a 12 weeks intervention) there was a marked reduction in M2-like macrophages and T-cells, suggesting that physical exercise is especially important for reducing inflammation in adipose tissue with very moderate reduction in body weight. Thus, this comparison indicates that energy restriction and physical exercise affect energy-related pathways as well as inflammatory processes in different ways, especially related to the amount and activity of macrophages. Whole genome gene expression profiles of PBMCs were evaluated as biomarker profile of diet-related health. In addition, PBMCs lipidome analyses were performed and integrated with the PBMC transcriptome. The aim was to study phenotypic flexibility by means of determining the whole genome transcriptional response and in human peripheral blood mononuclear cells (PBMCs) upon an Oral Glucose
Tolerance Test (OGTT) and a Mixed Meal Test (MMT) challenge before and after the energy restriction diet intervention.

We observed a change in genes and pathways at fasting and a consistent change in pathways such as OXPHOS and inflammation during the OGTT upon the energy restricted intervention when compared to the control group. These changes in PBMC transcriptome at both fasting and upon a challenge by a dietary intervention, that were paralleled by an improvement in health, illustrate the use of PBMCs transcriptome as biomarker for diet-related health. From our findings, we conclude that PBMCs transcriptome can be used as a biomarker for diet-related health, both at fasted and after a OGTT to test phenotypic flexibility.

PBMCs lipidome was also changed upon ER and integration of this data with the PBMCs transcriptome data have been performed in which subsets of metabolites correlated with each other. Further detailed analyses are still ongoing.

Workpackage 6 was divided into two parts: WP6A was concerned with maintenance of DNA integrity (led by Michael Fenech) and WP6B investigated the implications of DNA sequence and structural variation on the phenotype of the NutriTech participants.

The aims of WP6A here were to develop phenotyping methods to measure the Genome Integrity Maintenance Phenotype (GIMP) of the participants – this would examine how well each person was able to safeguard the DNA in their genome, especially in adverse conditions. A laser scanning cytometry (LSC) protocol for scoring micronuclei and the nuclear division index in cytokinesis-blocked lymphocytes has been developed for NutriTech and been published – this looks at damage to chromosomes and mitotic spindle apparatus. An LSC protocol for measuring γH2AX foci in buccal cells has also been published – this detects double-stranded breaks in the DNA molecules making up the chromosomes. The assays were then applied to lymphocytes from the NutriTech participants. There was a positive correlation between 8OHdG and γH2AX (R=0.52 and R= 0.56 for intensity and object measurements respectively which is expected given that 8OHdG is a measure of oxidative damage to DNA which also induces DNA strand breaks detectable by the γH2AX assay. There was a significant effect of the time when samples were collected from participants (P<0.01 for both measures of γH2AX and for the number of objects measure for 8OHdG) but no significant effect of whether they were in the weight loss group or acted as controls. There were no significant treatment-time interactions. The effect of time appeared to be due to a significant reduction in 8OHdG and γH2AX (P<0.05) the day following the first oral glucose tolerance test (W5D3T0) relative to the sample taken before the first challenge tests (W5D1T0).

In the WP6B part of the project we examined how both rare and common types of genetic variation affected the initial phenotypes of the participants, and their response to challenge tests. We used Illumina OmniExpress v24.10 arrays to carry out genome-wide genotyping for single-nucleotide polymorphisms (SNPs) – this detects common genetic variants, including many that have been identified in genome-wide association studies (GWAS) as being associated with relevant traits, such as BMI, T2D, fasting glucose, etc. These can be combined together into genetic risk scores (GRS) for the relevant traits. To detect potentially functional variants (including rare, coding sequence mutations, exome sequencing was carried out. There was no association of common genetic variants (individual SNPs, GRS, or AMY1/AMY2 region copy number) with either BMI or response to dietary intervention. However, when rare coding sequence mutations that are predicted (by SIFT and POLYPHEN2 programmes) to be harmful are considered, there is a clear interaction between the “allelic load” of mutations in monogenic obesity genes, and GRS. In NutriTech participants, background GRS makes a relatively greater contribution to BMI in people with
lower allelic load, than in those with more mutations in monogenic obesity genes. The same interaction is seen in the weight loss data after dietary intervention. This indicates that "genetic background" comes into play to a greater extent in the absence of major mutations. For the analysis of phenotypic flexibility, we analysed the effect of allelic load in key metabolic processes. It is notable that individual participants had almost identical responses to OGTT and MMTT in tests 3 months apart – exhibiting an unexpected degree of stability in their response to challenge tests. To examine whether this might reflect allelic load in key genes, we examined two functional areas, lipid metabolism and bile acid metabolism. Subjects with the highest number of deleterious alleles in lipid catabolism genes had different lipid profiles compared to volunteers with lower number of alleles. They had lower levels of free fatty acid acetylcarnitine and lower short/medium chain to long chain acylcarnitine ratio. Similarly, there were clear effects on bile acid responses to MMTT when participants were stratified for allelic load in key genes influencing this system. It seems clear that rare deleterious variants in genes affecting particular metabolic pathways may be key for explaining inter-individual responses to challenge tests.

The two objectives of WP7 were to store and share all project data and to integrate all methods and results into an overall assessment on phenotypic flexibility. Therefore, the NutriTech database, that was built into the Phenotype Data Infrastructure (http://www.dbnp.org/) was set-up that stores all information on study design, including clinical information, subject characteristics of all 72 volunteers and all the phenotypic data that was collected by the different NutriTech partners. All NutriTech partners had access to this NutriTech database so that every consortium member could use the entire dataset for their analysis. This NutriTech database will become publically available exactly 1 year after finishing the NutriTech project, to allow the public domain to evaluate and explore the most comprehensive study and dataset on phenotypic flexibility.

For the overall assessment on phenotypic flexibility, WP7 performed a multi-study analysis. Four studies studying phenotypic flexibility that are stored in the Phenotype Data Infrastructure were selected and evaluated. WP7 compared phenotypic flexibility of subjects with metabolic syndrome to healthy subjects. Metabolic Syndrome is a well-known health condition that is acknowledged as being a pre-state for chronic metabolic diseases, such as cardiovascular disease and type 2 diabetes. The output of this multi-study analysis was integrated with prior knowledge from public domain and structured in 3 different layers: the together formed a “health reference network”: 1) the very detailed molecular layer, 2) the aggregation of the first layer into >50 processes of phenotypic flexibility and 3) the aggregation of the second layer into 8 different “health domains” of phenotypic flexibility. The outcome of the entire NutriTech study results was integrated into this health reference network allowing the interpretation of the NutriTech intervention effect on molecules, processes and health domains affected in Metabolic Syndrome. It appeared difficult to identify major improvements at the whole population level by evaluating the single markers and single datasets. Yet, by evaluating the entire NutriTech dataset into the context of other studies and prior knowledge, WP7 concluded that caloric restriction improves beta-cell function, protein metabolism, lipokine and adipokine production and hepatic tissue control, thereby having an impact on pancreatic health, muscle health and adipose tissue health, which are major health domains that are compromised in Metabolic Syndrome. It also contributed to the identification of a practical biomarker panel of phenotypic flexibility. Biomarkers in the panel were studied for their long-term health impact by application in cohorts in WP8.

Overall, this WP has made significant contributions to the integrative analysis of the very comprehensive dataset of NutriTech by storing and sharing the complete dataset with the consortium and integration of
NutriTech results with other studies and prior knowledge from the public domain, confirming that phenotypic flexibility acts as a key factor in the human nutrition and health relationship as described by van Ommen et al. (Genes Nutr. 2014;9(5):423). The multi-study analysis showed indeed that the onset of many chronic metabolic diseases resulted from impairment or even loss of flexibility in parts of the system. The caloric restriction as applied in the NutriTech study improved the orchestration of several mechanisms and processes that contributing to phenotypic flexibility.

The objective or WP8 was to extend the findings from previous WPs and apply selected dietary and phenotypic flexibility biomarkers in cohorts. The importance and necessity of dietary biomarkers is well recognized in the nutrition field. Biomarkers provide objective measurements of dietary exposure, which may limit biases and errors often associated with the use of dietary questionnaires. A number of dietary biomarkers for sources of macronutrients were identified in the NutriTech intervention study (workpackage 1). Their application in epidemiological studies requires proper validation of these biomarkers in free-living subjects through the statistical comparison of the information obtained from dietary questionnaires with biomarker data. Two different studies were conducted in the European Prospective Investigation on Cancer and nutrition (EPIC) cross-sectional study for validation of biomarkers for intake of respectively plant and animal foods. Urine samples were analysed by high resolution mass spectrometry coupled to ultrahigh performance liquid chromatography and associations between intake of six foods of plant origin, three types of meats, and fish, measured with a 24-hour dietary recall or food frequency questionnaires were studied. A number of novel dietary biomarkers for coffee, tea, red wine, citrus fruits, chicken meat, red meat, ham and fish were identified and validated. In addition, the JINGO cohort was also used to validate certain biomarkers for chicken intake identified in workpackage 1 through NMR.

Regarding the practical biomarkers of phenotypic flexibility, we capitalized in acute and chronic interventions. Regarding the first, the postprandial phase is highly dynamic resulting from continuous metabolic adaptations that present a dramatic inter-individual variability, most probably reflecting an individual’s phenotypic flexibility. We examined this phase under two different challenges: a fat challenge (OFTT) and an oral glucose tolerance test (OGTT) in subjects of the CORDIOPREV Study and the results of these acute interventions as well as those from the chronic dietary intervention relevant to Nutritech are summarized here.

Initially, the postprandial plasma triglyceride (TG) response following a fat challenge among normal-weight, overweight and obese patients, according to their metabolically healthy or abnormal status, in a total of 1002 patients, with established coronary heart disease (CHD), participating in the CORDIOPREV clinical trial. The test meal consisted of 0.7 g fat/kg body weight (12% saturated fatty acids (SFA), 10% polyunsaturated fatty acids (PUFA), 43% monounsaturated fatty acids (MUFA), 10% protein and 25% carbohydrates). Serial blood test analyzing lipid fractions and inflammation markers (high-sensitivity C-reactive protein (hs-CRP)) were drawn at 0, 1, 2, 3 and 4 h during postprandial state. Both, TGs and hs-CRP were part of the practical biomarker panel to be tested in WP8. We explored the dynamic response according to six anthropometric/metabolic phenotypes: (1) normal weight, metabolically healthy; (2) normal weight, metabolically abnormal; (3) overweight, metabolically healthy; (4) overweight, metabolically abnormal; (5) obese, metabolically healthy; and (6) obese, metabolically abnormal. Our results demonstrate that metabolically healthy patients (groups 1, 3, 5) displayed significantly (p<0.001) lower postprandial responses of plasma TG and large triacylglycerol-rich lipoproteins (TRLs)-TG, compared with those metabolically abnormal (groups 2, 4, 6), independently whether or not they were obese.
Consequently, the area under the curve (AUC) of TG and AUC of large TRLs-TG were significantly (P<0.001) greater in the groups of metabolically abnormal patients (2,4,6) compared with the group of metabolically healthy patients (1,3,5). Moreover, metabolically abnormal subjects (2,4,6) displayed significantly higher (p<0.001) postprandial response of plasma hs-CRP than metabolically healthy patients (1,3,5). Therefore, these findings demonstrate the poor resolution of traditional measures of obesity as predictors of phenotypic flexibility. Therefore, more precise assessment, based on accurate measures of fat depots in the body and practical biomarkers, as proposed in Nutritech, are the best strategy for risk assessment and personalized treatment of obesity.

Once established the relevance of lipid metabolism and inflammation in phenotypic flexibility we move forward to investigate the contribution of two key factors: genetics and chronobiology. We have consistently found associations between single nucleotide polymorphisms (SNPs) of the circadian locomotor output cycles kaput (CLOCK) gene with cardiometabolic conditions such as obesity and dyslipidemia. Therefore, we proceeded to examine whether the chronic consumption (>12 months) of two healthy diets [low-fat (LF) diet and Mediterranean diet (MedDiet)] interacts with SNPs of the CLOCK gene (rs1801260, rs3749474, rs4580704) in order to improve lipid metabolism and inflammation status in CORDIOPREV patients. We found a significant gene-diet interaction between rs4580704 SNP and the LF diet. Specifically, major allele carriers C/C displayed a greater decrease in hs-CRP (p < 0.001) and a significant increase in HDL/apolipoprotein A1 ratio (p = 0.029) than minor G allele carriers (G/G + C/G). These findings suggest that rs4580704 SNP interacts with the LF diet improving inflammation status and dyslipidemia related with CHD and potentially reflecting a better phenotypic flexibility.

After the solidification of these findings related to lipid metabolism and inflammation both acutely and chronically, the next focus was on insulin-related homeostasis. Along these lines we investigated whether basal insulin resistance (IR) phenotype (muscle and/or liver) defines the effects of long-term consumption of the LF or MedDiet on tissue-specific IR and beta cell function in CORDIOPREV participants. At baseline, the patients were classified into four phenotypes according to the type of IR: (1) no IR; (2) muscle IR; (3) liver IR; (4) muscle + liver IR. The hepatic insulin resistance index (HIRI), muscular insulin sensitivity index (MISI) and disposition index were analyzed at baseline and after 2 years of follow-up. At baseline, 322 patients presented no IR (1), 106 presented muscle IR (2), 109 presented liver IR (3), and 105 presented muscle + liver IR (4). With both dietary interventions, HIRI decreased in all patients (p < 0.001) and MISI increased in muscle IR (2) and muscle + liver IR (4) patients (p < 0.01). Long-term intake of the MedDiet significantly increased the disposition index and insulinogenic index in the muscle IR (2) patients and the disposition index in the muscle + liver IR (4) patients, whereas the LF diet significantly increased the disposition index in the liver IR (3) patients. Therefore, both diets improve insulin sensitivity; however, we found differences based on basal IR phenotypes. Moreover, according to insulinogenic and disposition index data, a LF diet might be more beneficial to patients with liver IR (3), whereas patients with muscle IR (2) and muscle + liver IR (4) might benefit more from a MedDiet.

The translation of this research is to decrease the expression of CHD. Therefore, we investigated more in depth the relationship between anthropometric and metabolic measures and disease using as a surrogate carotid intima media thickness (IMT-CC). Thus, we measured metabolic markers of phenotypic flexibility and performed carotid ultrasound assessment in Cordioprev participants. Our data show that those with two or more metabolic abnormalities showed a greater IMT-CC than metabolically healthy individuals. Consistent with our previous observations, overweight and normal weight patients who were metabolically healthy showed a lower IMT-CC than the metabolically abnormal groups. When we evaluated only body weight independently of metabolic status, overweight or obese patients did not differ significantly from...
normal-weight patients in their IMT-CC. However, obesity was a determinant of IMT-CC when compared to the composite group of normal weight and overweight patients (all not obese). Therefore, in coronary patients, a metabolically abnormal phenotype is associated with a greater IMT-CC, and may be linked to a higher risk of suffering new cardiovascular events. Although the protection conferred in the IMT-CC by the absence of metabolic abnormality may be blunted by the presence of obesity.

In view of these findings we examined these variables in response to an OGTT. In a multiple linear regression analysis, only glucose concentration at 120 min, appeared as a significant independent contributor of IMT-CC (p < 0.001). Moreover, a multiple logistic regression and the area AUC of the ROC curve analysis showed a predictive power of glucose at 120 min to detect those CHD patients at the highest risk, defined as IMT-CC ≥ 0.7 mm (R(2) = 0.221; AUC = 0.761). Moreover, these effects were more significant in younger patients (<61 years old). Therefore, glucose concentration at 120 min could be a marker of CVD risk and impaired phenotypic flexibility.

So far we presented separately our findings related to lipid and insulin metabolism; however, phenotypic flexibility encompasses the homeostasis of whole body metabolism. In order to investigate the intersection between lipids and glucose we analyzed the postprandial lipemia response in CORDIOPREV participants according to the diabetic status. We studied the dynamic response in 57 non-diabetic, 364 prediabetic and 581 type 2 diabetic patients (T2DM). Additionally, the postprandial response was evaluated according to basal insulin resistance subgroups in patients non-diabetic and diabetic without pharmacological treatment (N = 642).

Prevalence of undesirable postprandial TG (>2.5 mmol/L at any time point) was 35 % in non-diabetic, 48 % in prediabetic and 59 % in diabetic subgroup, respectively (p < 0.001). Prediabetic patients displayed higher plasma TG and large TRLs-TG postprandial response compared with those non-diabetic patients (p < 0.001 and p = 0.003 respectively). Moreover, the AUC of TG and AUC of TRLs-TG was greater in the prediabetic group compared with non-diabetic patients (p < 0.001 and p < 0.005 respectively). Patients with liver insulin resistance (liver-IR) showed higher postprandial response of TG compared with those patients with muscle-IR or without any insulin-resistance respectively (p < 0.001). Therefore, our findings demonstrate that prediabetic patients show a lower phenotypic flexibility after a challenge, such as OFTT compared with nondiabetic patients. The postprandial response increases progressively according to non-diabetic, prediabetic and T2DM state and it is higher in patients with liver insulin-resistance. To identify this subgroup of patients is important to treat more intensively in order to avoid future cardiometabolic complications.

Having established these relationships between biomarkers, phenotypic flexibility and disease status we returned to investigate the influence of genetics on an individual’s risk. Specifically we examined whether tumor necrosis factor alpha (TNFA) gene variants (rs1800629 and rs1799964) were associated with inflammatory status. The hypothesis being that this locus could affect the rate of telomere shortening and its relation to cellular aging, an expression of poor chronic phenotypic flexibility. As in previous instances we used the Cordioprev Study. GG subjects for the SNP rs1800629 at the TNFA gene showed shorter relative telomere length and higher plasma levels of hs-CRP than A-allele subjects (p<0.05). Consistent with these findings, the expression of pro-inflammatory (TNFA) and pro-oxidant (p47phox and the gp91phox) genes was higher in GG subjects than A allele subjects (p<0.05). Therefore, subjects carrying the GG genotype for the TNFA rs1800629 SNP show a greater activation of the proinflammatory status than A-allele carriers, which is related to ROS formation. These ROS could induce DNA damage especially in the telomeric sequence, decrease the telomere length and induce cellular aging. Therefore, this locus could be a marker for poor phenotypic flexibility and the development of age-related diseases.
Finally, one of the objectives of Nutritech was to expand the relevance of the findings beyond White populations in Europe. For this purpose we examined the interaction of an S100A9 gene variant with saturated fat and carbohydrates to modulate insulin resistance in 3 populations of different ancestries. S100 calcium-binding protein A9 (S100A9) has previously been identified as a T2DM gene. Our aim was to replicate the associations between the S100A9 locus and insulin resistance and T2D in different cohorts and to initiate an investigation of potential interactions with the habitual diet. Therefore, we investigated the association of the S100A9 variant rs3014866 with insulin resistance and T2DM risk and its interactions with diet in 3 diverse populations as follows: the CORDIOPREV, the GOLDN (Genetics of Lipids Lowering Drugs and Diet Network; n = 818), which involved North American non-Hispanic white adults; and Hispanic adults who participated in the BPRHS (Boston Puerto Rican Health Study; n = 1155). Our results indicated that T carriers presented a lower risk of T2DM than CC carriers (pooled OR: 0.714; 95% CI: 0.584 0.845; P = 0.002). In all 3 populations (CORDIOPREV, GOLDN, and BPRHS), we showed a significant interaction between the rs3014866 SNP and dietary SFA:carbohydrate ratio intake for the homeostasis model assessment of insulin resistance (HOMA-IR) (P = 0.028 P = 0.017 and P = 0.026 respectively). CC carriers had a significantly higher HOMA-IR only when SFA:carbohydrate intake was high (P = 0.045 for the CORDIOPREV, P = 0.033 for the GOLDN, and P = 0.046 for the BPRHS) but not when SFA:carbohydrate ratio intake was low.

In summary, the minor allele (T) of the S100A9 variant rs3014866 was associated with lower T2DM risk in 3 populations of different ancestries. Note that individuals with the high-risk CC genotype may be more likely to benefit from a low SFA:carbohydrate ratio intake to improve insulin resistance as evaluated with the use of the HOMA-IR and thus phenotypic flexibility.

WP9 – Harmonisation and dissemination

NutriTech has developed a comprehensive series of methods to quantify phenotypic flexibility, based on integrated use of emerging and established technologies, some developed/refined for NutriTech. These Standard Operating Procedures have been assembled into one “biomarker paper” describing the various biomarkers, their mechanisms, their relation to phenotypic flexibility and their predictive value for health-related outcomes. This biomarker paper will be published as a scientific review, and subsequently become also available on line at the NutriTech website and the NuGO SOP-Portal. These biomarker methods are indeed already used for example by some major food companies, by 7 European Research Centres using MRI protocols, but also overseas like in South Korea research centres and the USDA Research Center at Davis CA. Challenge tests as biomarkers are also more and more accepted. These methods are one of the key elements for getting a harmonised approach when conducting Phenotypic Flexibility Research and allowing scientists conducting such research to compare and exploit all results made available. In this context of harmonisation, NutriTech has also established contacts with key scientific journals to encourage them to adopt the policy of requiring standardised open access of relevant data and results in the appropriate data depositories, in line with the vision of the Functional Genomics Data Society (Ref: http://www.gmed.org/). The JPI program Enpadasi (http://www.enpadasi.eu/) continues to maintain the database used by NutriTech.

Most dietary biomarkers currently have been identified on the basis of our knowledge of food compositions by using hypothesis-driven approaches. However, the rapid development of metabolomics resulting from the development of highly sensitive modern analytic instruments, the availability of metabolite databases, and progress in (bio)informatics has made agnostic approaches more attractive as shown by the recent identification of novel biomarkers of intakes for fruit, vegetables, beverages, meats, or complex diets.
Moreover, examples also show how the scrutiny of the food metabolome can lead to the discovery of bioactive molecules and dietary factors associated with diseases. However, researchers still face hurdles, which slow progress and need to be resolved to bring this emerging field of research to maturity. NutriTech contributed to advancing knowledge in this area of food intake metabolomics, partnering with the World Health Organisation (WHO) International Agency for Research on Cancer (IARC) in organising the 1st International Workshop ‘The Food Metabolome And Biomarkers For Dietary Exposure – Metabolomic Approaches For Biomarker Discovery, Validation And Implementation’ (Ref: ‘The food metabolome: a window over dietary exposure’ (Scalbert A. et al., Am J Clin Nutr, 2014 Apr 23;99(6):1286-1308)). The purpose of this workshop was to convene for the first time key experts in metabolomics, nutrition and epidemiology in order to define the most promising and shortest routes to mine the food metabolome and identify biomarkers needed to better understand the role of the diet in disease aetiology. Key recommendations made during the workshop included more coordination of efforts; development of new databases, software tools, and chemical libraries for the food metabolome; and shared repositories of metabolomic data. This should lead to major progress toward a better understanding of the complex interactions between diet and human health. This workshop also set the basis for setting up mid-2015 ‘The Food Biomarkers Alliance’ (FooDBall) (Ref: http://foodmetabolome.org/) consortium funded by the JPI-HDHL; an initiative aimed at identifying and quantifying dietary biomarkers in order to improve the capabilities of nutritional assessment and research.

The concept of Phenotypic Flexibility and the progresses of the NutriTech research and related findings have been advertised in many ways such as papers, in Dissemination Regional Workshops organised as part of key conferences (2015 FENS (Federation of European Nutrition Societies) in Berlin, Germany, EB15 (Experimental Biology, Boston, United States of America, and ICoFF (International Conference on Food Factors), Seoul, South Korea) or with talks in relevant international conference, leading to interaction with Scientist interested in the fields and contributing to the harmonisation of the research being done. More information on NutriTech can be found at: http://www.nutritech.nl

Potential Impact:
NutriTech has pursued two main objectives, one scientific (the design and validation of new biomarkers of health), and one technological (the validation and integration of new technologies in nutrition research). Both are discussed here related to their impact.

Biomarkers
NutriTech has on the one hand expanded the concept of challenge tests (biomarkers of stress response mechanisms) by broadening the topic beyond the classical oral glucose tolerance test to all relevant processes involved in phenotypic flexibility. On the other hand, we have assessed the complexity of the integrated molecular physiological response to a series of practical biomarkers, together quantifying the important phenotypic flexibility processes related to health outcomes.

The project progress was accompanied by a strong and open dissemination, actively sharing methods as they were being developed. This resulted in global collaborations, for example with EHWA Womens University, Seoul South-Korea (Dr Oran Kwon). Dr Kwon’s team has performed a series of human intervention studies using challenge tests as biomarkers. Also, The USDA Western Human Nutrition Research Center at Davis CA (Drs John Newman and Lindsay Allen) has adopted the mixed meal tolerance test in a 400 subject cross sectional phenotyping study with extensive health quantification.
related to the challenge test.

The phenotypic flexibility biomarkers also raised considerable attention with Food Industry. This resulted in the “PhenFlex” project of TNO with five major food industries (Nestlé, Abbott Nutrition, FrieslandCampina, DSM and Dupont (former Danisco) on the topic of these biomarkers, closely collaborating with NutriTech. Over a period of three years, this project explored a number of aspects, in human studies. i.e. the ranges of phenotypic flexibility biomarker responses in a healthy population, and the comparison of healthy and type 2 diabetic subjects in the OGTT and Mixed Meal Challenge Test (i.e. identical to the ones used in NutriTech). The PhenFlex consortium had regular meetings with the NutriTech project, and full open access to each other’s data was agreed upon. The PhenFlex project is continued for a second phase, where a proof of principle human intervention study will be executed with the “lessons learned” in NutriTech and PhenFlex, both in a classical RCT manner, and in a more personalized approach, exploiting the sensitive inter-individual variations observed in the NutriTech intervention study.

Finally, the first steps of exploiting the phenotypic flexibility concept in healthcare were made alongside the project. The CordioPrev study from NutriTech partner Cordoba demonstrated in three recent papers how differences in response to OGTT and mixed meal challenge tests could classify patients in subgroups with differences in disease onset and progression, and differences in reaction to various healthy diets. Also, TNO exploited very similar subtyping in newly diagnosed type 2 diabetes patients to advise them one of six different lifestyle related treatments, based on subtyping of pancreatic beta cell function, liver insulin resistance and muscle insulin resistance. The first results indicate that this personalization provides better results that the standardized care.

Innovative Methods
The NutriTech approach in the human intervention study was, to our knowledge, the most extensively phenotyped and genotyped human intervention study ever performed. Technologies applied were whole exome sequencing, DNA-damage evaluation, transcriptomics of PBMC, muscle biopsies and adipose biopsies, dedicated plasma proteomics, various methods of metabolomics, intestinal microbiome quantification, whole body MRI, where applicable during three different challenge tests (OGTT, mixed meal challenge test, also combined with a physical exercise challenge).

Each of the technologies was assessed for its robustness and meaningful application in human intervention studies. More importantly, two translations were made. Firstly, what is the added value of integration of the methods and data into a “systems approach”, and secondly, how can these usually complicated technologies deliver cost-effective and practical biomarkers.

- Integrated approaches
The integration of the data of the NutriTech study posed some major challenges. A strategy was developed having three aspects
1) overall analysis of the major outcomes of the study, based on the most obvious outcomes.
2) in depth analysis of specific processes from an integrated molecular physiological view (examples: glucose metabolism, carnitine metabolism, bile metabolism, inflammatory processes) – each of these analyses resulting in scientific publications
3) building of a “systems map of phenotypic flexibility”, bringing together the main outcomes of 1) with the detailed evaluations of 2).
These approaches appeared to be time consuming as methodologies were available only in a very generic way, and needed to be further developed and fine-tuned within the consortium. A major lesson of this project is thus the need for innovative data evaluation tools beyond the current associative methods.

- Alternatives to Average Approaches
Interestingly, a major result of the extensive geno- and phenotyping of the 72 study subjects before and after the dietary intervention was that although the study population was highly stratified and the main outcome (average weight loss) was accurately following the predicted outcome, major inter-individual differences were observed. In fact, having enormously data-intense results, we could observe a multitude of different reactions to the intervention. These ranged from simple gender differences to strong variation in lipid depletion in the pancreas, with corresponding differences in improvement of insulin sensitivity. Apart from the large amount of additional work this created for the consortium (and a fair part of this is still “work in progress”), it raised the awareness that the classical study design and evaluation focusing on main average outcomes is strongly underutilization the potential information of these studies, once the effect is measured in detail. Alternative evaluation methods were included, e.g. linking genetic variations to phenotypic subgroups, correlating various observed subtypings to unravel causal mechanisms, and designing systems approaches for data evaluation to facilitate multi-technology / multi-omics layer evaluation from a physiological perspective.

- Open access and Open research
The NutriTech consortium agreed to strongly promote open access and data sharing of all study data. To this extent, all study data were captured in a single data repository, the nutritional phenotype database. This database was originally designed by NuGO and brought to maturity by various initiatives and consortia, while it is now positioned as part of the Joint Programming Initiative (the Enpadasi project). This database allows web-based sharing and once the complete curated dataset is finalized, the study data will become open access.

In the meantime, NutriTech has actively stimulated open collaborations. The above mentioned PhenFlex consortium is an example, while other collaborations (the Norwegian MyoGlu project, the South-Korean example, the use of NutriTech exome data in rare genetic variant research, etc.) were shaped during the project. Upon finalizing the project, the wealth of data and the opportunity to continue data exploration and exploitation urge open collaborations. Consortium members are actively establishing these. NutriTech maintains a “Knowledge management Committee” for this purpose.

Dissemination activities

A series of dedicated dissemination activities were organized. Four dedicated symposia in 2012 (Madrid), 2015 (Boston and Seoul) and 2016 (Lisbon) focused on interacting with academic and industrial stakeholders.

Exploitation of results

The results of NutriTech are exploited by food companies. Challenge tests to quantify health effects of foods or food compounds are now recognized to be a sensitive biomarker. A number of food companies are currently using these in their research and development portfolio. On a broader view, the concept of
phenotypic flexibility as underlying basic mechanism, and the opportunities to optimize this by dietary products (both generic and subgroup-specific) is also exploited by industry.

The biomarker panel developed by NutriTech has been the subject of an SME-brokerage event organized by NutriTech in 2016, but despite extensive PR this meeting was cancelled due to lack of interest. In other words, SME could not see a specific exploitable value in this panel.

Partner Biocrates is proceeding with the valorisation of a food intake quantification biomarker package within their metabolite quantification technology. Also, a number of patents are being written on specific aspects of metabolome-based biomarker profiles by NutriTech partners.

List of Websites:
The address of the project public website, if applicable as well as relevant contact details.
www.nutritech.nl

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