Diogenes Final Publishable Executive Summary

Summary description of project objectives

The rapid increase in the prevalence of obesity and associated co-morbidities is a major global health problem. In Europe obesity directly consumes about 5 % of total health care budgets. Of greater societal concern is the rapid increase in childhood obesity afflicting all European countries, which needs immediate action, as was urgently requested by the European Council of Ministers in 2002. Although in principle a simple energy imbalance problem, obesity is a puzzling condition. Whilst susceptibility to obesity is determined largely by genetic factors, the current obesity epidemic is significantly influenced by adverse lifestyle factors. Given our genetic background it is an increasing challenge for humans to self-regulate food intake under current environmental circumstances. This worrying trend should force the scientific community to expand its research efforts using a wide variety of innovative approaches.

Diogenes aims to be just such an innovative effort, including all those disciplines and stakeholders who can contribute to achieving a better understanding how this disease can be prevented and treated from the dietary perspective. The primary focus of Diogenes is an investigation of dietary macronutrient components that will facilitate the prevention of weight gain and regain, studying specifically the role of the Glycaemic Index (GI) of carbohydrate and of a high dietary protein content for enhancing satiety. The objective is to examine interactions between the dietary components and genetic and behaviour factors.

The seven main objectives are closely related to the seven research lines

RTD line 1: Obesity and macronutrient composition of the diet
RTD line 2: Obesity and gene-nutrient interaction
RTD line 3: Obesity, genes diet at the population level
RTD line 4: Obesity, consumer attitudes and behaviour
RTD line 5: Obesity and food technology
RTD line 6: Central data hub Facility
DIDT line 7: Dissemination, Innovation, Demonstration and Training

Partners involved

At the end of the fifth project year the Diogenes consortium includes 31 partners across 14 countries. The consortium is made up of world-class centers in diet and health studies, epidemiology, dietary genomics and food technology. It also includes 3 major food industrials and 5 Small- and Medium-Sized Enterprises (SMEs). The dietary intervention study, which is coordinated by RTD1 in collaboration with RTD2 and RTD4, will cover centers in 8 of the countries. The project is coordinated from the University of Maastricht. The Executive Director, Professor Wim HM Saris, is supported by the Project Secretariat, which includes two project managers and a project secretary. Contact details of Prof. Saris, the project secretariat as well as more information on the Diogenes Project and the 31 Diogenes partners can be found on the Diogenes website at http://www.diogenes-eu.org/

Work performed, results achieved and end results

RTD line 1: Obesity and macronutrient composition of the diet

To examine critically the mechanistic impact of changes in macronutrient composition on weight control, a large long-term randomised, parallel dietary intervention study is undertaken in whole families suffering from obesity or overweight. The overweight-obese parents follow an 8-week weight loss intervention using a Low Calorie formula diet (LCD). The families in which at least one parent achieves a weight loss of minimum 8% is randomised to one of 4 ad libitum diets with

either high or low GI, combined with high or normal protein content or to a control diet (following current official guidelines). This part of the study takes place in the following 8 different countries across Europe: Denmark, the Netherlands, UK, Germany, the Czech Republic, Bulgaria, Spain and Greece.
In the first project year the detailed procedures and Standard Operating Procedures (SOP's) were completed and ethical approval from the local ethical committees was obtained. In the second project year, two of the eight centres, Denmark and The Netherlands, started the recruitment and screening of families in April 2006. By November 2006 the recruitment and LCD intervention phase were finalized. In these two centres the ad libitum intervention lasted for 12 months where families obtained free food from an on-site supermarket for 6 months, and received dietary instruction for a further 6 months. The supermarket setup, which allows for a close computer based monitoring of the families purchases, was set up by spring 2006 in time for the randomised supermarket based intervention which was completed in June 2007. This part of the study addresses the efficacy of the intervention. The last Danish and Dutch study participants completed the subsequent "6 month dietary instruction only" period in November 2007. In the other 6 centres, the instruction based randomised intervention was initiated during the second project year. In these centres the families only receive dietary instructions during the 6-month ad libitum intervention phase. Thus, this part of the study assesses the relative efficacy and feasibility of diets varying in protein content and GI in preventing weight gain and regain. The outcome should be influential in shaping dietary guidelines and recommendations for persons who are already obese or are at risk of developing obesity. Recruitment, screening and the LCD period were finished by end of April 2007 whereas the 6-month dietary intervention study in whole families was completed by beginning of year 4. By end of year 4 all 8 centres have completed the follow-up visits and as such the dietary intervention study is concluded.
The huge amount of research data collected during the various clinical investigation days, obtained through questionnaires and food diaries have been entered continuously into the Data hub throughout the third, fourth and fifth year. Monitoring and coordination of data collation is led by the RTD1 and RTD6 leaders, who in turn update the involved RTD2 and RTD4 leaders on the progress regularly.
The delicate handling of the thousands of samples, their shipment and subsequent storage has been planned and discussed in detail among the RTD1 centres and has been topic in many internal training sessions. The shipment plans have been communicated broadly among the involved RTD line 2, 4 and 6 partners as well. The shipments of thousands of samples from the 8 RTD1 a location towards one central place of storage was coordinated by RTD1 centre LIFE and the Gentofte Core Lab and executed successfully by the 8 centres.
Sample analysis started in the 3rd year, and was completed in June 2009. Data derived from sample analyses has been entered into the datahub on an ongoing basis, and by the June 2009, all the data from the clinical analyses performed at Gentofte Core Lab and at LIFE, Copenhagen is now available in the datahub. Here, the results where merged into the existing data from the dietary intervention study. In addition the RTD6 has developed a variable list that is be placed at the website www.diogdata.dk, in which information on the collation status (when is data cleaned and by whom) of the different variables entered into Epidata including the clinical samples. All the clinical samples will furthermore be formally and rigorously cleaned by the authors that use them in their scientific publications. Hence a process have been completed where all authors of key publications have been asked to give contribution to the data cleaning process for the data covered by their synopses.
The existing food composition tables used for calculating dietary intake in the Diogenes intervention study lack information on Gycaemic Index (GI) and Glycaemic Load (GL) of foods and therefore are not useful to calculate the GI and GL of the 3-day food diaries recorded by adults and children in the study. To this end GI values have to be incorporated into the food composition tables. Measured and published GI values are certainly available for a number of foods, but much of the work has been carried out in non-European countries and there is a paucity of measured GI values for commonly consumed European foods. The Establishment of a Glycemic Index table and incorporation into existing food tables is a challenging task that has been coordinated by the RTD1 centres HNR and UM and the method by which GI values were

assigned to the different foods, was developed by scientists from HNR. The establishment of such a European Glycemic Index table is currently ongoing.
A Biobank was set up at Maastricht University during the 4th project year where all spare samples are stored for later analysis with novel techniques. All samples are stored under optimal conditions, and are labeled with anonymous id-codes identifying project, research center and subject ID. To use the samples from the Biobank at any time point in the future, a synopsis defining aim of the project, the samples needed, the analyses planned and the plans for publication must be presented to and approved by the Diogenes Project Board. During the 5th project year a subset of stored samples has been shipped to the Netherlands Metabolomics Centre (NMC) at Leiden University. The samples will undergo a multivariate statistical analysis (PLS-DA, O-PLS, ASCA, MSCA) including thorough model validation to obtain biological interpretable metabolite patterns. Global metabolite profiling by GC- and LCMS and targeted profiling of low-molecular weight lipids (LCMS) and endocrines (LCMS) has been started. These data collections will be subjected to multivariate statistical analysis, including thorough model validation to obtain correlations between dietary interventions, metabolite patterns, gene expression and anthropomorphic changes. Metabolic pathway analysis will be carried out to interpret changes in metabolic signatures and effects on physiological endpoints. These activities are planned for Q3 2010 and later as part of the post Diogenes work program based on the extended Consortium Agreement between all partners involved.
As by the end of year 5, the three methodological papers on the RTD1 study have been written, approved by the Diogenes Consortium and published in the Obesity Reviews as a special supplement publication. The key results have been presented at various obesity conferences, including the European Conference of Obesity (ECO) in Amsterdam 2009, and a total of 5 core papers representing the main results from the dietary intervention study in obese families have now either been submitted to various journals, or they are currently in their last phase of revision, before being finally submitted early in year 2010. Furthermore, 3-4 other non-core manuscripts based on RTD1 data are in preparation and an extensive list of additional manuscripts are in the pipeline. All 8 partners involved in the RTD1 study have agreed to take part in the Diogenes extended consortium agreement on a voluntary basis, thus ensuring the full and complete reporting of the many and important scientific results that were successfully achieved in the study.

RTD line 2: Obesity and gene-nutrient interaction
Within the framework of the DiOGenes project, RTD line 2 focuses on gene-nutrient interaction associated with changes in body weight and metabolism in order to guide a diet-based control of weight.
One of the main goals is to highlight factors (genes, transcripts or proteins) with either levels changing regarding the nutrient content of the diet or able to predict the ability of one's individual to lose weight under energy restriction, or to maintain the reduced weight.

RTD line 2 addresses these questions with 3 levels of investigation:

- Genomic DNA, by investigating DNA polymorphisms in selected candidate genes.
- Messenger RNA, using gene profiling of subcutaneous abdominal adipose tissue (AT).
- Plasma proteins, by studying the plasma peptide and protein levels using a priori or novel peptidomics and proteomics approaches.

The **genetic** part of RTD line 2 focussed on assessment of gene/nutrient interaction in relation to weight changes and related traits through genotyping of selected candidate genes. The work was based on analysis of two populations: EPIC (DK, UK, FLO, RIVM, POT) and RTD1 collections. At the beginning of Diogenes project a first list of 12 genes which was further enlarged to 74 genes was set up jointly with RTD1 and RTD3 for association studies. The final SNP list was ready at the beginning of year 3, comprising 768 tag-SNPs that: 1) tag the common genetic variation of each of the 74 candidate genes 2) have potential or proven functional implications 3) have been extensively studied in the past for relationship with weight, or have shown highly significant associations in recent studies or have a high likelihood of genotyping success rate. During this time, genotyping started on two EPIC collections (UK and DK).

By year 4, 614 SNPs were correctly genotyped on the 12,000 samples of the 5 EPIC collections and data was transferred to the central data hub in Copenhagen. RTD1 DNA samples were extracted. During year 5, 669 SNPs were genotyped on 1342 samples from RTD1 population. This year was devoted to the statistical analyses. The loss of interest from IG delayed these analyses. It was decided to change the responsibility for the analyses to the project Coordinator, the RTD1 coordinator, RTD3 and the RTD2 coordinator together with Datahub. Numerous synopses were produced. For EPIC data, none of the statistically significant gene/SNPs remained significant when corrected for multiple testing. However, based on significance levels of FTO, a key gene in weight control, 2 genes were selected for analysis in combination with AT gene expression to evaluate a potential functional effect of these SNPs. For RTD1 data, a model is built to assess the effect of SNP interaction with dietary protein or glycaemic index.

The **transcriptomic** part of RTD line 2 was held by INS.
It was based on AT gene expression methods including:

- microarray gene expression profiling to predict weight changes.
- microarray gene expression profiling to find out weight control and nutritional molecular biomarkers.
- quantitative RT-qPCR to validate the genes selected on the basis of prediction algorithms and weight or nutritional biomarkers.

As a first step AT samples from the 5th framework Nugenob project were used. This program consisted in a 10-w low calorie diet (LCD) with 2 dietary arms, one with low fat /high carbohydrate diet (LFD), the other one with moderate fat/low carbohydrate diet (MFD). AT transcriptome was studied across these diets with the idea to set up analytical strategies for the search of weight changes predictors or biomarkers as well as nutritional markers.
With the completion of the dietary intervention study in RTD line 1, samples and subjects' information were available to RTD line 2 at the beginning of year 4. When INS has received and prepared all RTD1 AT total RNA samples, Diogenes subjects selection started based on selection criteria common to both transcriptomic and proteomic RTD2 groups. 205 subjects from 4 diet groups across the DiOGenes study met selection criteria together with good quality blood or fat biopsy samples available.

For weight changes prediction:
Nugenob AT samples of females who lost less then 4 kgs were compared at baseline (before starting LCD) to those who lost between 8-12 kgs. The goal was to build a classifier that will be able to determine weight loss after LCD using only data collected during the initial exams.
Despite the AT transcriptome was very similar in both groups the prediction rate of 73% suggested that predicting response to diet for an individual might become more accurate.

To identify predictors of weight loss during the LCD phase of the DiOGenes program, AT gene profiling for both CID1 and CID1b (2 days after the LCD started) was performed on 2 groups of subjects determined on the basis of weight loss: "good responders" being subjects losing more than 13% of their initial body weight and the "poor responders" being those losing about 9% of their initial body weight. The results obtained by the different classifiers showed promising results with a significant shift compared to previous results obtained in the framework of the Nugenob study with prediction accuracies up to 91% whatever the time point considered.
To identify predictors of weight changes after the LCD phase of the DiOGenes program, AT gene profiling for both CID1 and CID2 was studied on subjects selected based on weight maintenance and weight regain during the 6 month weight maintenance period; The genes expressed in all subjects across both time points were used to estimate the accuracy of various predictive models. Running the model gave better results at CID1 than CID2, indicating that changes in weight can be predicted at baseline based on the AT gene expression profile.
A shortlist of genes that play a key role in the various prediction models was validated using quantitative RT-PCR.

For the search of weight control and nutritional molecular biomarkers:

Changes in gene expression of Nugenob AT samples of women during either the LFD or MFD showed that energy restriction impact more than diet composition on the AT transcriptome. However, using classification trees, a small subset of genes was found and validated as up-regulated during MFD.
Gene profiling data of DiOGenes AT samples was used to find weight or diet-related genes. Weight maintainers and regainers were selected using a selection criteria (S score) that reports weight changes during 6-m maintenance phase, taking into account the weight lost during LCD. This S score was used to classify subjects. After exclusion of the 10% extremes, 6 lowest S and 6 highest S subjects were selected in each dietary arm as “unsuccessful” (those who regained the weight they had lost) and “successful” (those who did not regain weight) subjects.
Accordingly to the study on Nugenob samples, it was found that weight changes impact more than nutrient composition of diet on AT transcriptome.

When comparing AT transcriptome of successful subjects to those from unsucessful subjects, most of the differential genes are mainly regulated regarding the change in weight after caloric restriction. Most of these genes encode proteins involved in inflammation, cell proliferation and apoptosis.
In order to find genes with expression changes depending on either dietary protein levels or GI, another set of subjects was selected with minimal weight variations during the stabilization phase to avoid confounding effects of weight changes. Statistical analyses showed that genes are mainly modified by GI rather than by protein dietary levels. GI variations led to modification in genes related to metabolism while protein variations led to cell signaling related genes. A selection of these genes was validated by RT-qPCR.

Proteomic studies started by year 3.
A list of targeted proteins/peptides being set up, relevant analytical assays have been compared to ELISA for the analysis of targeted proteins and steroid hormones in plasma samples of CID1, CID2 and CID3 at UM.
Due to insolvency DBVN terminated its activity from month 41 and PS has taken over the peptidomics analysis using a new quantitative peptidomics workflow. PS uses a new quantitative peptidomics workflow based on labelling peptides in plasma with heavy-isotope stable tag. The techique, namely tandem mass tags (TMT®) with electrospray ionisation tandem mass spectrometry (LC-ESI MS/MS), is more precise in quantitation and with a general higher sample throughput.
NEST has developed a method and workflow enabling medium-throughput qualitative and quantitative analysis of human plasma proteins using commercially available human plasma. The optimized workflow was applied to pilot samples from DiOGenes.

During year 4 and proceeding year 5, among the 205 RTD2 eligible subjects defined above, 97 successful and unsuccessful subjects from 4 diet groups, covering all 8 RTD line 1 centres, were selected for targeted protein and peptidomics study.
The blood samples have been shipped from central lab to UM, DBVN (to PS later) and NEST, respectively.

UM has tested plasma samples of CID1, CID2 and CID3 of the 97 “successful” and “unsuccessful” subjects using multiplex immuno-assays in different companies and by in-house ELISA assays. 20 proteins and steroid hormones were identified as predictors of weight changes during weight maintenance period, and 21 factors as biomarker to successful or unsuccessful weight maintenance. 13 proteins and steroid hormones were identified as biomarkers of diet protein or GI. 50% of the factors identified were confirmed in blood samples from 48 Diogenes male subjects.

The comprehensive profiling of precursor peptides was performed at PS in the plasma of the 97 subjects described above. It was completed at baseline CID1 and CID2, as well as after the oral glucose tolerance test at CID1 and CID2. Statistical analysis showed that, based on their plasma peptide profile at CID1, it is possible to distinguish good and poor responders to LCD. Biomarker

candidates are now incorporated in a multi-marker test for predicting the outcome of dietary interventions. IP for this test will be filed.

For the quantitative plasma proteomic, CID2 and CID3 blood samples from a subset of the female subjects selected as described above were analyzed. NEST focused on 2 diets only (low protein/low GI and high protein/low GI) for logistic reasons. Seventy-five proteins matched all the criteria of data inclusion. Among these eligible proteins, 26 differentially expressed proteins were identified including 5 proteins as biomarkers for successful or unsuccessful weight maintenance.

All experimental designs involved the same subject selection to enable data integration in between omics studies. Altogether these genetic, transcriptomic and proteomic data allowed DiOGenes to focus on target genes/proteins that will be tested on the whole DiOGenes population in the coming year.

RTD line 3: Obesity, genes diet at the population level

With access to large long-term prospective cohorts across Europe with clinical and nutritional data, DiOGenes has a unique opportunity to identify the role of key dietary factors and of gene-nutrient interactions associated with changes in body weight and waist circumference. The 5 partners representing the EPIC (European Prospective Investigations into Cancer) cohorts with 145.000 subjects in 5 European areas (Florence, Italy; Potsdam, Germany; Bilthoven, The Netherlands; Norfolk UK; Copenhagen/Århus, Denmark) have collaborated on defining strategies for analysing pre-existing food record data with the primary focus on Glycemic Index, Glycemic load and protein intake (including different types of protein), as well as other summary information about habitual food intake. These and other parameters, such as other lifestyle factors, familial predisposition, psychosocial and socio-economic factors, etc. will be carefully analysed for their association with changes in body weight and body fat distribution. During the second project year summary measures of the key dietary factors and the food compositions constituting these factors was optimised, including completion of a database of GI/GL for the EPIC study. Further, follow up measures was completed in the last of the 5 cohorts and selected baseline and follow up data were integrated. A strategic analytical plan and a list of specific aspects to be analysed were prepared addressing the role of various dietary aspects (including GI and protein) in relation to change in weight and body fat distribution. Extensive data analyses have been carried out during the 3rd project year to examine GI and protein intake in relation to weight change and waist change in the 6 centers. This was based on a common methodological guideline developed for this purpose. Decisions were taken on cleaning the data exclude missing values and building the statistical models.

In addition to the EPIC cohorts a Danish and a Finnish twin cohort (GEMINAKAR and FinnTwin16, respectively), with data from more than 2500 twin pairs have been included to address the quantitative genetic, shared and non-shared environmental influences on key dietary habits and on the development of obesity. During the first year initial statistical analyses were performed, followed during the second year by assessment of quantitative genetic, shared and non-shared environmental influences on the key dietary habits and on development of obesity. Analyses addressing the extent to which the key dietary factors can account for the environmental influences on development of obesity while controlling for the quantitative genetic influences on the dietary habits were initiated. In 3rd project year the analyses of the association between the dietary components and obesity phenotypes were finalized. Further combined analyses of the genetic and environmental influences on bread intake and bread choice in the Danish and Finnish twin cohorts were completed. In addition analyses of correlations between MZ intrapair differences in dietary intake and obesity phenotypes were completed by the end of 3rd project year. Three new tasks related to eating and food related behaviour, control of genetic variation, and analyses of metabolomics data were formulated and initiated in the beginning of 2008. In the Danish twin sample genotyping was performed for the FTO rs9939609 polymorphism and three SNPs near the MC4R gene. During the fifth project year the analyses of the polymorphisms in relation to dietary intake and anthropometry were performed. Initial analyses of the reduced outcome of the NMR based metabolomics data in the Danish twin cohort were

conducted during the forth project year and analyses of the full spectra NMR based metabolomics data were completed in the fifth project year.

DNA has been extracted from the EPIC cohort samples to be genotyped by the RTD2 line partners. The gene and SNP selection, which was coordinated by RTD3, was finalised during the 3rd project year yielded a list of 75 genes. Genotyping started during the 3rd project year and was completed in the 4th. The "analytical strategy" working group which consisted of RTD2, RTD3 and RTD6 members, discussed and explored several methods to analyse the data in the context of gene-diet interaction and weight gain. In the 4th project year the analytical strategy group finalised its analytical strategy, which was implemented during the analyses of the available data. During the final project year the analyses of associations between the genetic variants, dietary intake and weight and waist changes as well as possible gene-diet interactions were performed by the Datahub.

Based on the participating EPIC cohort and the results of the analyses of associations between key dietary factors and changes in weight and waist a model of predicting weight gain was constructed during the fifth and final project year. The model follows the concept that has been applied for the German risk score (www.dife.de). The model contained only independent significant predictive modifiable life style variables with the focus on diet. Subjects having gained weight more than 1% per year were selected as cases. This means, that we predict the probability to be a case, p. e. if a person of 60 kg gained more than 600 g/year or a person with 80 kg more than 800 g/year. The beta-coefficients of the model were transferred into a point system. Subsequently, the model parameters respective the points were used to predict attributable risk and the development of weight gain if a dietary factor is changed. In this stage, genetic variation was not included due to lack of findings useful for prediction. Variations in the FTO gene seems to be the most promising candidate, however, it is currently not clear how to integrate these findings obtained from a case-cohort approach into the prediction model. Taking the current and future trends into account a prediction of the prevalence of obesity in the cohort for the year 2015 were constructed. Two different models were used; one assuming a linear increase over time and one assuming a levelling off of the increase with time. It appears that the simple linear model lead to a prevalence that is similar to the status in the US at the current time period with an obesity prevalence of above 30%. Taking the EPIC-Potsdam data with repeated measurement of weight recording over time it appeared that during the last years weight increase is much less than in the initial period of the observation indicating that over time the dynamic of weight increase is reduced and that less weight increase in seen in the population nowadays. This levelling off of weight gain could be simulated by a log transformation. It appeared that a log transformed model fit much better to the EPIC Potsdam data than a linear model. The log transformed model, if applied to all EPIC centers participating in Diogenes, predicts also a considerable increase in prevalence of obesity in 2015 but much less as seen in the US today with an estimate of the prevalence of obesity of 25% for most European study populations.

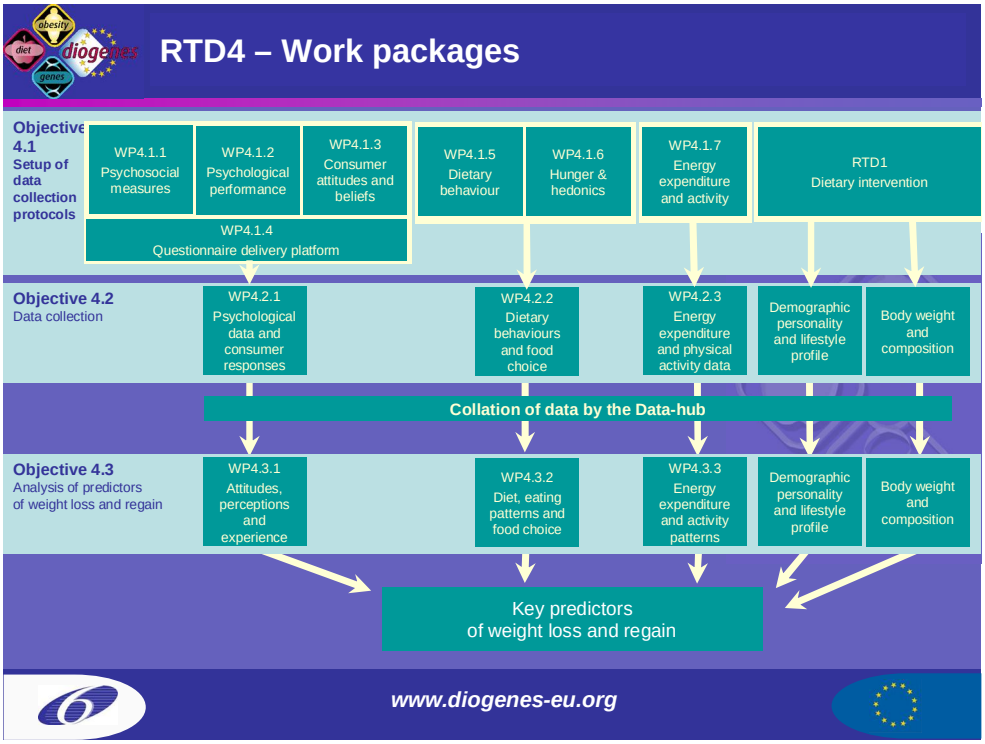
The results of the analyses in Diogenes RTD line 3 will be been written up in manuscripts and submitted for publication in international peer-reviewed scientific journals. By the end of the project 13 papers had been published, 2 papers had been accepted for publication, and 23 papers were in review or in preparation.

RTD line 4: Obesity, consumer attitudes and behaviour

The main objective of RTD4 was "to assess the lifestyle and psycho-social aspects of food intake, in order to identify key psychological/behavioural predictors of weight gain for use in diagnosing risk of weight gain and for better matching diets to consumer needs". The majority of the work undertaken by RTD4 in the Diogenes was facilitated through data collected from two cohorts – the RTD1 dietary intervention cohort and a group of slimmers from the commercial slimming organisation (and partner in RTD4) Slimming World. In the most comprehensive examination to date of the relation between diet and phenotype in the control of body weight, RTD4 aimed to identify key psychological/behavioural predictors of successful and unsuccessful weight control,

for use in diagnosing risk of weight gain and for better matching diets to consumer needs (figure 1).

Figure 1 Overview of the overall RTD 4 workplan



The work undertaken during the first year of the project was related to the development of a range of tools and procedures for assessing the different psychological and behavioural aspects expected to play a role in the risk of weight gain (objective 4.1). During the first project year, questionnaires were selected and developed for assessment of psychosocial measures determining expectations, the impact of modest attempts to control weight in a family environment, weight control behaviours, emotional state and quality of life, as well as attitudes, perceptions, beliefs and barriers to successful weight control. All questionnaires were translated into the local languages of the 8 RTD1 clinical centres and set up in a web based data capture tool, which participants completed online, while attending the examinations at the clinical centres. The web-based Questionnaire Delivery Platform (QDP) was developed by RTD4 and implemented in the 8 RTD1 clinical centres for the collection of questionnaire data. Procedures to determine energy expenditure using double-labelled water, physical activity using the IDEEA movement meter and appetite responses to a test–meal were also developed.

During the 2nd project year data collection was initiated in all the eight clinical centres. The data collection was closely monitored though various tracking procedures. Further training sessions and visit to each of the RTD1 centres assured the proper implementation of the procedures related to the various measures, and support to the clinical centres. During this year the development of strategies for data analyses was initiated. Further, a system for integrating the food recoding data from the eight clinical centres was developed and implemented. In addition, during the second year of the project preparations were made to collect data from the second cohort of subjects - members of the commercial slimming organisation, Slimming World.

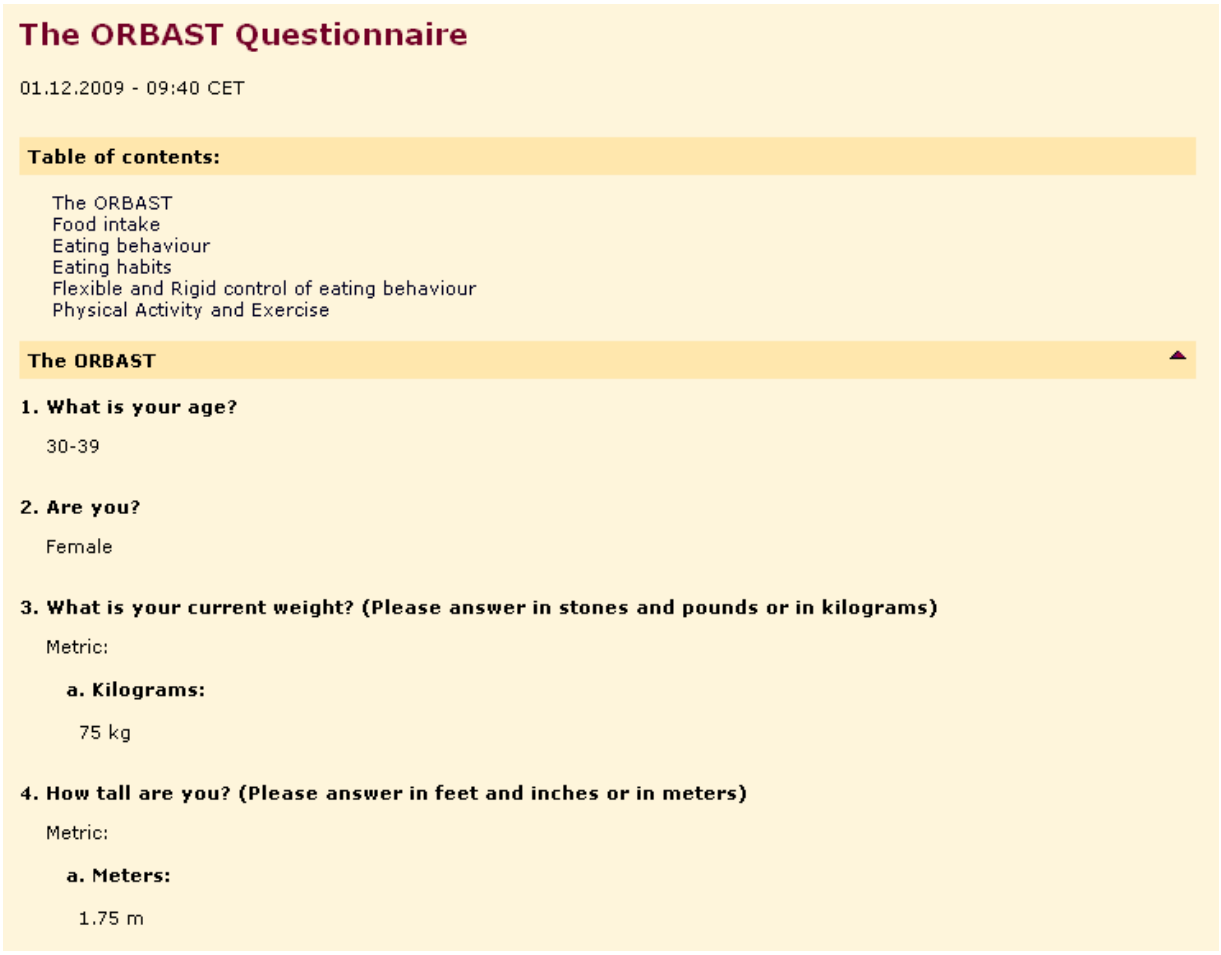
Throughout the 3rd year of the project a large part of the work undertaken was related to collection and monitoring of psychological data, consumer responses, data describing dietary behaviour and food choice as well as energy expenditure and physical activity data. During this time, RTD4 worked collaboratively with the Datahub and RTD1 to develop plans and a process for handling and accessing the data being collected in both the RTD1 centres and the Slimming World cohort. A list was then developed within RTD4 of all the relevant variables from which summary variables and subscales could be generated from the datahub. In addition, work on analysis and interpretation of the data collected was initiated during year 3, and was continued in year 4.

The collection of RTD4 data in the RTD1 dietary intervention trial was successfully completed during year 4 of the project. A lot of collaborative work was undertaken during year 4 between the Datahub and the RTD1 teams cleaning the data and conducting preliminary data checking analyses. All data were in the Datahub by the end of the fourth year and analysis of the RTD4 data commenced at this point. Summary variables were incorporated into the Datahub to facilitate more efficient analysis by the RTD4 partners and these were refined and rechecked during the preliminary analysis stage. Analysis of the EDQ data, food choice and test meal data and physical activity data, looking at change over time, were prepared and reported on by the end of the fourth year, in a series of deliverable reports. In addition, the psychosocial and attitudinal data were being investigated, with analysis of baseline data complete by the end of the fourth year.

During the fifth project year, concentrated work on development of the ORBAST prototype was the primary objective of RTD4. All data collected for RTD4, along with any relevant RTD1 data (demographics, weight measures etc), were collated and used in the modelling analysis for the ORBAST development. Analysis of predictors of weight loss and its maintenance which indicate key risks of weight gain and behavioural pathways was conducted. The outcomes of these analyses were used together with a detailed examination of studies conducted elsewhere in the literature, to identify the key variables to measure in the ORBAST tool in order to give consumers some indication of key risks of weight gain and behavioural pathways to recommend to avoid weight gain. In addition to the data analysis, work on ascertaining the current state of the art in online tools designed to identify and quantify risk of weight gain was undertaken.

This state of the art report was combined together with the information gathered in the reviews and assessment of the literature available and the results of the Diogenes predictive analysis a roadmap for the ORBAST was compiled. The roadmap formed the starting point for the technical and user-interface specifications for the ORBAST prototype. The front end of the ORBAST is the user interface and asks a number of questions in a shorter, but similar format to the QDP (figure 2).

Figure 2: The ORBAST user-interface (screenshot)



The ORBAST Questionnaire

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The ORBAST

1. What is your age?

30-39

2. Are you?

Female

3. What is your current weight? (Please answer in stones and pounds or in kilograms)

Metric:

a. Kilograms:

75 kg

4. How tall are you? (Please answer in feet and inches or in meters)

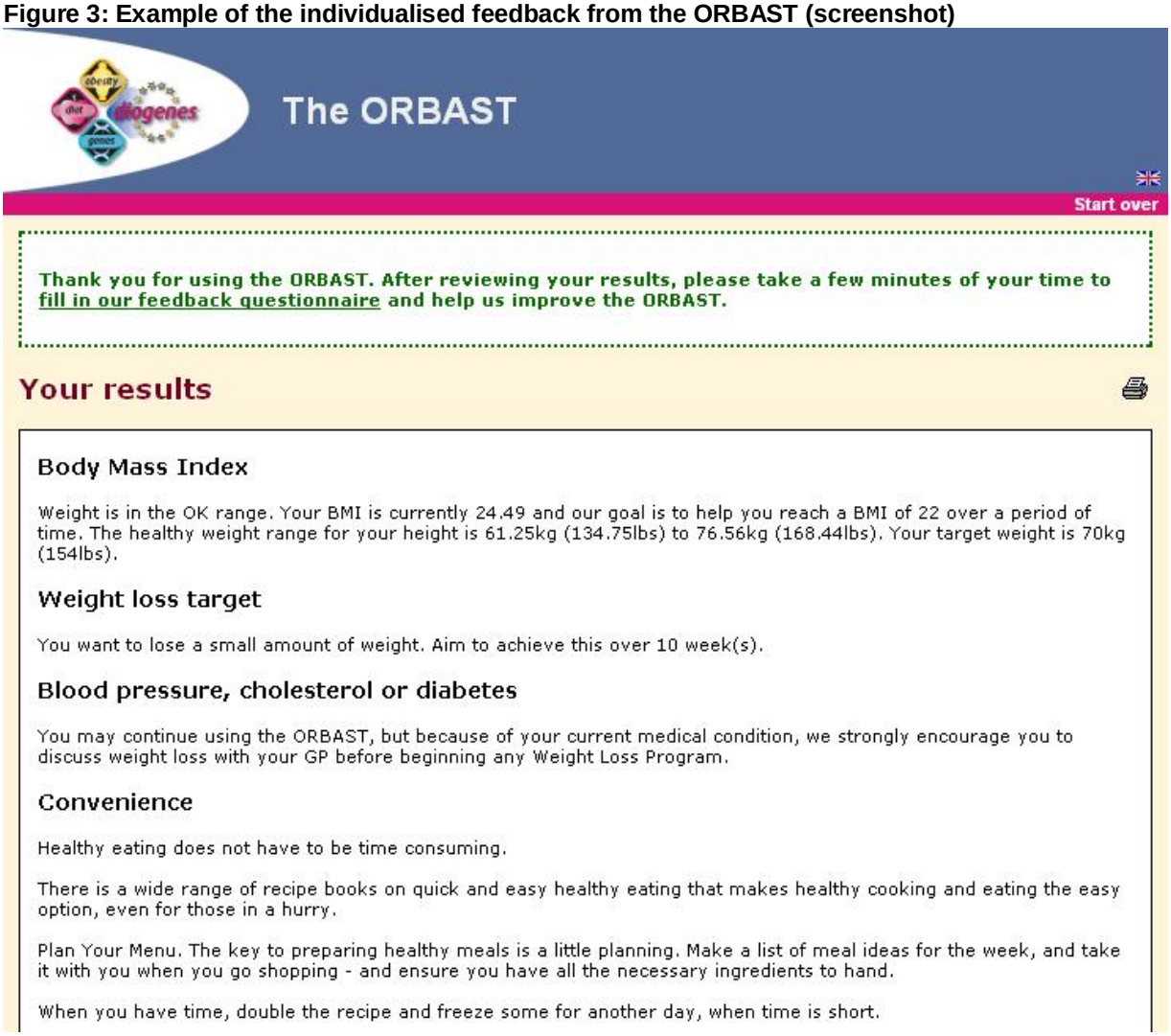
Metric:

a. Meters:

1.75 m

Behind this lies the weighting factors estimated form risk associated with the specific behaviours, outlined in the user interface. The applying the subject's score of their own questionnaire to the weighting factors generates a behaviour profile and risk assessment, which is converted to an individualised lifestyle report for each user of the ORBAST (Figure 3).

Figure 3: Example of the individualised feedback from the ORBAST (screenshot)



The ORBAST prototype has been developed into an evidence based software tool that:

1. Describes key behavioural correlates of weight gain and regain in the DiOGenes and Slimming World cohorts as reference populations.
2. Describes to the individual (through a questionnaire-based tool) their own profile of weight control risk behaviours.
3. Allows the individual to compare their current behaviour with those who are successful or unsuccessful at maintaining weight loss and avoiding weight regain, so they can establish where they sit in terms of likelihood of gaining weight in the future.
4. Provides links to reputable organisations who give advice on and implement solutions to behaviour change and lifestyle approaches to weight control.

Users of the software tool will be provided with an Individualized report with the range, group average and the individual's score or value for each of the behaviours identified and access to information on how to implement the advice given by the ORBAST.

RTD line 5: Obesity and food technology
Food technology plays an important role in relation to the treatment and prevention of obesity. The aim is to develop foods which the consumers will like and prefer, but which will at the same time limit intake through enhanced satiety signals. This requires a combination of skills and disciplines seldom found in industry and science.

During the first project year, a panel of subjects was recruited and preliminary experiments were conducted in relation to identification and evaluation of physiological, oral and olfactory triggers involved in regulation of food intake behaviour. At the same time a dedicated olfactory set-up was created for the Diogenes project. In addition, efforts were devoted to the development and testing of foods and condiments expected to have food intake regulatory properties. Particular focus was placed on development and testing of products with high protein content or low GI levels. The product development and testing phase was initiated immediately after the start of the project and resulted in first prototypes of high-protein sausages. Screening of sources for resistant starches (RS) to be used in low GI bread and pasta development was also conducted in the first year.

In the second year the examinations and initial data analyses were completed. Further studies were conducted addressing the effect of MSG+IMP-5 on macronutrient preference and on satiety, palatability, energy and substrate oxidation of a high-protein meal and subsequent food choice, as well as analysis of fat/fatty acid triggers in eating, shamfeeding, and control conditions. From autonomic physiological responses in relation to food intake and satiety, the most relevant characteristics were heart rate and salivary alpha-amylase. These can be applied in a very straightforward way for subject characterization and selection, or in satiety and appetite profiling studies.
Consumption behaviour and attitudes related to oral anatomy and physiology, and sensory sensitivity to chemical tastants was also tested. Chemosensory threshold measurements and sensitivity for selected odours were tested in relation to food intake/satiety. Improved products with high protein content (sausages) and low GI (pasta) were developed, tested and ready for further testing within RTD-line 5, and one high protein product was delivered RTD-line 1 for the ad libitum intervention study.

Product development, testing of tailored food products, triggers and biomarkers that may influence food intake behaviour, satiety, energy expenditure, or blood hormones continued during the third year. This part of the study aimed at identification of novel food ingredient systems, which may induce satiety while remaining in the preferred sensory range. High-protein sausages and low GI pasta developed in RTD-line 5 were tested in trials in which satiety, energy expenditure, and blood hormones were measured. Particularly high-protein products showed an effect on markers for satiety.
Research regarding the effect of complexity in aroma quality in relation to satiation and food intake was completed and one flavour concept, complexity in aroma quality, was integrated in a real meal. Using complex instead of simple aromas provided more short term satiating effects.
Markers for satiety were investigated based on previous pilot study showing that salivary peptide patterns revealed a number of peptides that consistently varied with hunger. A validation study on these salivary peptide patterns performed in different age, BMI and gender classes revealed components that were related to a disturbed energy balance.
Another high-protein product, meatballs, were developed and tested on consumers for satiating properties and liking with good results. Two different products with RS, bread and pasta, were also developed and tested with good results. Processing conditions were shown to greatly influence starch resistancy.

In year four, tests were conducted on effects of a combination of bioactive components added to a 20% energy restricted diet on energy expenditure and satiety compared to a control diet in energy balance. A combination of bioactive components added to a 20% energy restricted diet resulted in higher energy expenditure and satiety compared to a control diet in energy balance. A combination of bioactive components may thus facilitate adherence to an energy deficit diet by sustaining satiety and energy expenditure. Testing of complexity in aroma qualities showed that a

complex aroma was able to significantly induce feelings of satiation compared to placebo single-component aroma.
The experimental work on development and testing of high protein meat products, products with resistant starches, and triggers used in products and condiments was completed. Final optimisation of high-protein sausages was conducted based on experience from production in large scale facilities in the previous period. Reduced GI-pasta was produced in an industrial environment and tested for organoleptic properties. Possibilities for commercialisation were investigated. Triggers were included in the tests and also tested out in a whole meal test for subject responses, see above. A food concept regulating food intake behaviour may contain different combinations of high protein products, resistant starch products, capsaicin containing products, and green tea.

In year five, final analyses of results have been conducted, and findings gained through previous years were presented at a special DiOGenes satellite symposium in conjunction of the ECO-conference.

Papers for publication in international journals have been prepared, and presentations at scientific conferences have been held regularly throughout the project period. In total three PhD's directly related to work conducted within DiOGenes were defended publicly during the project.

RTD line 6: Central data hub facility
By the start of the project a centralised data hub was established to ensure that all data generated in relation to the project are optimally integrated, stored, secured, and documented. The data hub has developed SOPs for data collection, documentation, cleaning and transfer.
During the second project year the DataHub collaborated intensively regarding the intervention study with RTD1 and RTD4 on implementing the strategies for data transfer and monitoring the progress though data monitoring. Further, an on-line randomisation program was implemented in the clinical RTD1 centres and the data hub further interacted with RTD2 and RTD3 on data integration and initial analyses.
During the third project year the main practical activities have been concentrated on receiving data from the intervention study involving data from RTD1, 2 and 4 and to integrate them into a common structure. Data concern measurements gathered during the clinical investigation days, questionnaire data, data obtained from food diaries, data acquired from sample analysis. There is an ongoing production of summary statistics for RTD1 and RTD4 in order to update these partners regarding the progress. In addition RTD6 providing training and has been interacting with the RTD lines regarding standard analytical strategies for the available datasets, as well as providing ad hoc assistance in relation to more specific analyses. The RTD6 coordinator participates in several working and writing groups with statistical expertise and in the analytical strategy group regarding the analysis of the gene-diet analyses together with the involved RTD2 and RTD3 partners.
During year 4 there are two main studies that involved data from several RTD partners and the Data Hub has used its main effort on these two studies. The first one is the cohort study based on 5 EPIC cohorts and the genotyping of a sub sample of 12.000 selected in a case-cohort design. The second one is the intervention study involving data collected by the clinical centres and the Core Lab.
During year five the database for the intervention study (RTD1+2+4) was completed with the inclusion of Double Labelled Water, final food diary data, and remaining data from analysis of urine samples. The genetic data has been integrated in the database. A more elaborate metadata description of the database has been developed and disseminated to all partners via the website www.diogdata.dk. Thorough data cleaning of anthropometrical, blood and urine measurements has been undertaken.
The statistical analysis of the intervention study with respect to dietary factors has been completed and has resulted in submitted manuscripts. The DataHub has in particular contributed with analysis accounting for family structure and drop-out.

Many resources were used this year in collaboration with RTD1-4 to conduct statistical analysis of gene-diet actions. In relation to the EPIC cohort studies 10 synopses were provided with results of the statistical analyses of gene-diet analyses performed by the DataHub. In relation to the intervention study gene-diet data are ready for analysis and an analytical strategy has been developed.

The data hub will continue in future years to host and integrate all data from the project, ensuring data documentation and back up, and will further be available for consultancy and collaboration in relation to optimising data analyses.

RTD line 7: Demonstration, Innovation, Dissemination and Training

The DIDT line has the generic objective to communicate knowledge from the Diogenes consortium and programme, so that it can lead to benefit for the consumer in alleviation of the obesity problem, advance the body of scientific knowledge, and spread use of Diogenes' specialist expertises and techniques inside and outside the consortium. The work-packages Innovation, Dissemination and Training, working closely with RTD lines 1-6 in a horizontal way, facilitate delivery of these end-points for the benefit of the whole project.

In relation to *innovation*, the first year has been devoted to building the necessary organisations and processes. A process for managing IPR across the project has been designed. One of its tasks of Year 2 was the review of patenting opportunities in the RTD programmes, and development of initial exploitation strategies. An internal team of the partners Unilever, Nestle and Hill have been formed to review the innovation potential in the RTD programmes and how it can be optimised. The team was supplemented by a new partner who joined the consortium at the start of Year 2 (Kraft) This team, together with representatives from the RTD lines, some external advisers (including members of the External Advisory Board (EAB)), with inputs from the patenting review and from the consumer/market obesity environment, considered the range of possibilities for delivering real consumer benefits from the Diogenes programme, and drafted a commercialisation strategy accordingly.
A workshop has clarified the sectors, beyond the food industry alone, which could potentially exploit Diogenes findings. The potential has been demonstrated for commercially available consumer research to provide important guidance on the environment in which new knowledge from Diogenes must be translated into consumer benefit. These inputs have informed an initial commercialization/exploitation strategy, for the foods and other relevant sectors.
At the end of the 3rd project year 2 patents have already been filed within RTD5.
During year 5, all RTD lines have been very actively involved in the data collation and data analysis stage. As more results emerged within Diogenes, it became more transparent if certain findings were exploitable or patentable.
In year 5, candidate biomarkers have been found that could be included in a diagnostic test for predicting successful weight loss and maintenance after a dietary intervention phase, and patent protection will be obtained for these biomarkers. It is expected that more patentable and exploitable findings will emerge out of the Diogenes project in particular from the RTD line 2 field of genomics and proteomics, after the official end of the project
In addition, scientific knowledge and "know how" was generated extensively during the 5th project year. These were presented at scientific meetings, including the Diogenes satellite at ECO2009.
In RTD 4 the ORBAST prototype has been developed, a tool that can subsequently be developed for consumers and health professionals, which can measure in terms of risk of weight gain or regain. Additionally, RTD1 made a new a GI-database. A new methodology has been developed for consistent assignment of GI values to foods across the five European databases used in the Diogenes intervention study.

During the first project year major effort was devoted to *disseminating* the goals and plans of the Diogenes project. Several editorial articles introducing the project were published in well-established international journals. A website www.diogenes-eu.org was launched, with both public and project members' pages. The public pages present the project objectives, the contractors involved and co-ordinator contact details, news, and links to related Sixth Framework

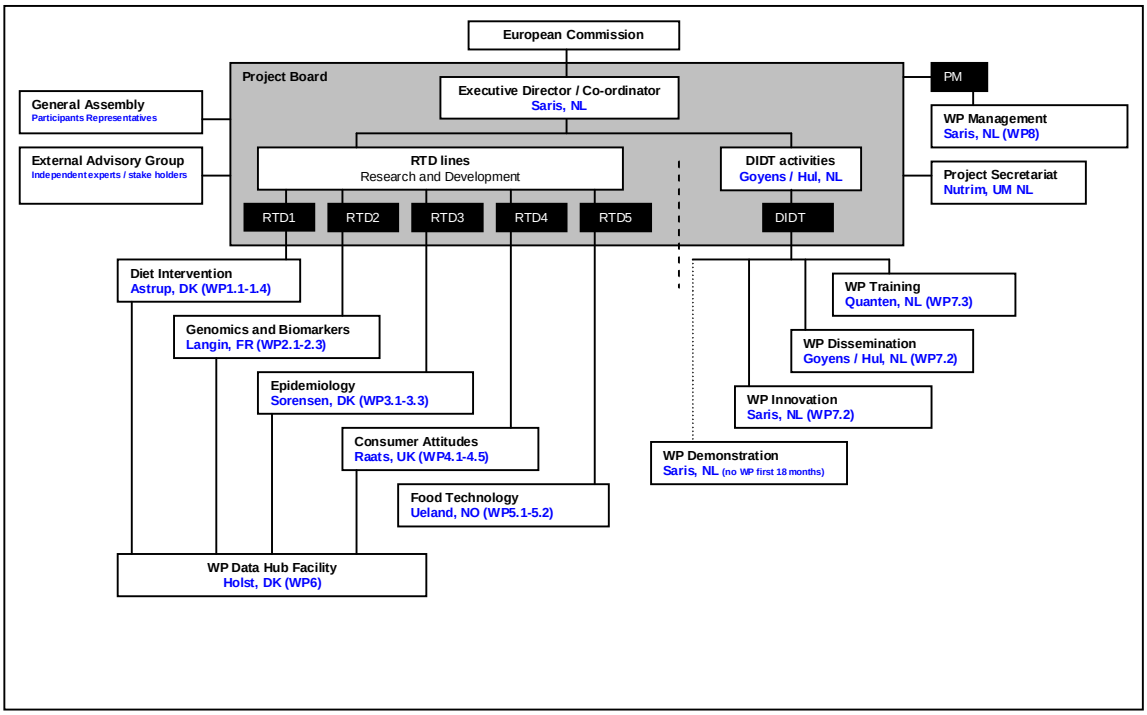
projects funded under the Food Quality and Safety Priority. They have also provided information on the RTD1 Dietary Intervention Trial for subjects interested in participating. In addition articles on the project were presented by various other web-sites including Cordis, Food Navigator and partners' own web-sites. The project has been presented at several national and international scientific and thematic conferences. They include the European Congress of Obesity in Athens, June 2005 in a session dedicated to 5th and 6th framework EU programs addressing obesity, and the 1st Obesity Europe Conference, Brussels, June 2005. A 'tool kit' for all the partners to use in ongoing dissemination of the project has been developed including: a brochure and a flyer, a slide pack, model press releases, Q&A, project logo and design elements, and a SOP giving guidance on good dissemination practices. Through the informal network CommNet, contact has been established with disseminators of other Food Quality and Safety FP6 projects, to explore opportunities for useful joint dissemination activities, and to share best practices.
In Year 1 *Dissemination* concentrated on launching the project. In Year 2, with no real results available yet, work has focused on keeping stakeholders aware of the project and its steady progress, and on initiating some activities for delivery later in the project. For ongoing communication, the web-site has been further developed (eg. with video-clips, news of the RTD 1 trial, etc); a 6-monthly Newsletter has been launched; posters have been displayed at a number of scientific meetings.
Partners in the RTD 1 trial have held media events to boost recruitment, and Sponsor Days to keep their foods suppliers informed.
In year 3 Diogenes has received media attention throughout Europe via a film report broadcasted on the EuroNews channel. A Diogenes Food/Industry workshop was held, hosted by Kraft. The main conclusions of this workshop were made public by means of a press release and through an article that was published in March 2008 in the journal Food Science and Technology. The website was used for ongoing communication. The Diogenes Newsletter has appeared twice in the 3rd and 4th project year. In year 4 the Diogenes film recording the human and organisational challenges of the RTD 1 trial was completed and launched during the fourth project year with an accompanying press release. A second press release announcing the disclosure of preliminary results at ECO2008 was distributed. Preparations for the Diogenes satellite at ECO2009 were initiated during the 4th project year.
The main dissemination activity of year 5 took place in the first half of 2009 and was directly related to the Diogenes satellite (May 4-6) at ECO2009 in Amsterdam. All possible efforts were made to disseminate the study results of the 5 RTD lines as widely as possible. The scientific community that was present during the meeting was informed through posters and presentations of Diogenes and external researchers. Diogenes also attended a number of events and conferences in year 5. In addition, the general public was reached through various media channels. Press releases were issued prior and during the meeting to draw the attention of the international media community.
To generate scientific knowledge in order to enhance the current understanding regarding the prevention of obesity and weight (re)gain, presentation to the research and (para)medical community through professional peer-reviewed publications in scientific journals and scientific congresses has been continued in year 5 and again several manuscripts have been published or accepted for publication. More manuscripts are being prepared at the moment, for submission within the coming year.
Throughout the 5 years of Diogenes, much effort has been put in communications in order to influence consumers beneficially on overweight- and obesity avoidance.
This has been successfully achieved by means of:
- Close working relationships with the scientific community and external stakeholder groups;
- Informing scientific community, nutritional and medical community, industry and popular media;
- Organisation of and participation in conferences, meetings & events;
- Identifying "hot-topics";
- Maintaining sponsor relationships;
- Dissemination via public side of the Diogenes website;
- Diogenes movie, regular newsletters;
- Satellite meeting at ECO2009;

These communications will be continued in the near future, mainly by presentations of former Diogenes scientists and through the Diogenes website which will continue to stay online for a number of years.
Diogenes also attended a number of events and conferences in year 5.

In the first project year, the training effort within Diogenes has focused on technical skills, processes and standards necessary for execution of the various parts of the programme by the RTD lines. A number of multi-partner training workshops have been delivered for this purpose. A priority for years 2-5 would be to disseminate specialist Diogenes skills/expertises beyond the consortium itself to potential users in the wider scientific world, esp. young scientists, in the form of learning packages.
As in year 1, the priority in year 2 has also been on training skills, processes and standards necessary to deliver the Diogenes program, with the RTD lines as main drivers. These activities, mainly intra- or cross-RTD line workshops, ensured that the skills necessary for execution of the Diogenes programs were embedded in the institutes and persons who are required to practice them, and established a uniform and, where possible, recognized (eg. GCP) quality standards. This training approach has also stimulated a culture of integration and team-working across the consortium.
During the 3rd year a “Genomics in obesity course” was organized by partner INSERM for young researchers active in Diogenes and the EU funded projects HepaDip and NUGO.
A statistical training was organised by RTD6 for all RTD1 centres to train necessary skills for the analyses of data. Two media training sessions were given for young Diogenes researchers. The first
PhD thesis on Diogenes data was successfully completed and publicly defended in June 2008.
Training activities in the fifth and final year of the project consisted of some activities that have been formulated in previous years and still need to be executed or that are ongoing.
A workshop, which was initially scheduled for the fourth project year, was organised in Month 57 by projects Diogenes, Hepadip and Interact. The workshop, entitled: “Translation of genetic variation to gene function in biology and Epidemiology” has provided participants with knowledge in the field of modern population-based molecular genetics in the area of obesity and related fields, with a focus on aspects relevant for interested non-geneticists. In total 30 people participated in the workshop, of which 11 participants from the Diogenes project.
Ongoing activities within WP7.3 have been research visits between partners, to strengthen the coordination of work and processes between partners. In 2009 another 3 PhD students have completed the work on their Diogenes-based thesis (RTD 3 and RTD 5). More PhD (a total of 17) will continue with their work, being guided and supervised by the senior scientists within the project, also after the official end date of the project.

Management
Management and coordination of the project and of the consortium has been an ongoing task during the 5 years of the project. The Project Board, consisting of the Project Coordinator, RTD Line coordinators and the management team has been the final decision-making body within the project. All major strategic decisions, including the decisions with regard to project management have been made by the Project Board. Maastricht University was designated by the consortium to act as coordinator and has carried out these activities over the 5 years of the project.
Responsibilities have included the organisation of meetings to harmonise activities between the various work packages, the allocation of received payments made by the Commission, reporting and communication within the European Commission, preparation of work plans and budgets per year, incorporation of new partners and overall management. The Project Board has also promoted gender equality within and outside the project, and coordinates training and teaching activities.
The different RTD Lines have been coordinated by the respective RTD Line Coordinators which has ensured that the objectives of the project have matched the goals of the work plans.

The Diogenes organisational structure during the period funded by the European Commission is presented in the scheme below:



The meeting structure was established at the beginning of the project and has continued to be used throughout the project. Every project year, two consortium meetings have been organised in different locations in Europe. At these meetings all partners of the project have gathered for meetings within research lines, as well as meeting between research lines, and for overall consortium and general assembly sessions. In addition, 2-3 separate meetings for the Project Board per year have been organised. An External Advisory Board (EAB) was been established during the first project year. The EAB members were invited to participate in the consortium meetings and joined one of the yearly Project Board meetings.

The members of the Diogenes External Advisory Board (EAB)

Representative medical field: Prof.dr Hans Hauner
Member of European Association for the study of Obesity (EASO) director, Else Kröner Fresenius Zentrum für Ernährungsmedizin, TU München Munich - Germany

Representative scientific aspects related to food industry: Nico van Belzen (PhD)
Executive Director of the International Life Sciences Institute (ILSI) – Europe Brussels – Belgium

Representative nutrition field: Prof.dr Jo Hautvast;
Retired from Wageningen University, :Wageningen Centre for Food Sciences the Netherlands (Chairman of EAB)

Representative political field: Jules Maaten;
Member European Parliament. Liberal Group in the Parliament (Dutch liberal party VVD) member of committee for the Environment, Public Health and Consumer Affairs Strasbourg – France / the Netherlands.

The project website (www.diogenes-eu.org) was established in the project preparation phase, but was re-launched with the new Diogenes logo and layout during the first months of the project, and has continuously been extended and updated. Part of the website is a members section, which has served as the website as well as a high-level intranet facility. The website was continually updated on an ad hoc basis.

Ethics
The study is performed according to the latest version of the Declaration of Helsinki, the UN Convention on the Rights of the Child and the Current International Conference on Harmonization (ICH) guidelines. Wherever possible, the study is performed according to the current Good Clinical Practice (GCP) guidelines. All regional Ethics Committees for the RTD1 and RTD5 centres have approved the study.

Gender
A gender officer was elected during the first project year. The participants agree to promote gender equality in the different sections of the project as well as in the composition of the teams responsible for the different committees. All participants are initially sensitised to aspects of gender bias within the project. All partners have a policy in place for equal opportunities that collectively covers all employees. The majority of the partners have an “Equal opportunity officer” or a contact person for equal opportunity issues who would be consulted to optimise the equal opportunities within the consortium. The Project Board takes care of the gender issues in the project and has the authority to reject proposals from partners if the gender equality is not taken into account, i.e. if the explicit justification for a departure from equality is insufficient. The RTD line coordinators are responsible for the gender issues within the section programs and look for gender equality. Each RTD line will report back annually to the Project Board about gender issues. The gender officer has attended the Network meeting on Gender aspects in Food Quality and Safety Research. In January 2007 the Diogenes officer was invited to participate in the Gender Basic Expert Meeting entitled “Promoting integration of the Gender Dimension in (basic) life sciences research. The consortium has been kept updated on the gender strategy by the gender officer. Furthermore the Diogenes Newsletter, edition Autumn 2007, contained a 2 page text on “Gender aspects in Diogenes” written by the Diogenes’ gender officer and a presentation of seven women that are active at the various research or management positions in the Diogenes Project. In order to increase our understanding of differences between genders in food related behaviour, two book chapters have been compiled in year 3 and in year 4. The findings presented in these chapters by the gender officer have implications for both setting up of dietary regimes and understanding why compliance with dietary advice may be low if gender is not taken into consideration. Gender has been an important factor in designing interventions and experimental studies, and results from these studies justify the attention on gender as can be seen in the publications and presentations from DiOGenes.