

Embargoed until 00.01am Wednesday 14 May 2008



Diet, Obesity and Genes

Arne Astrup on behalf of The DiOGenes*

Department of Human Nutrition, Faculty of Life Sciences, University of Copenhagen, Denmark.

Background

The rapid increase in the prevalence of obesity and associated co-morbidities is a major global health problem. In Europe obesity is directly responsible for more than 5 % of total health care budgets, and indirect costs to the management of type 2 diabetes are at least of the same order. Of major concern is the rapid increase in childhood obesity afflicting all European countries, which needs immediate action as urgently requested by the European Council of Ministers. Although in principle a simple energy imbalance problem, the factors leading to obesity are complex. Whilst susceptibility to obesity is determined largely by genetic and intrauterine factors, the current obesity epidemic is heavily influenced by adverse lifestyle factors. Given our genetic background, it is essentially infeasible 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.

Aim of DiOGenes

The overall general objective of the DiOGenes integrated program is to reduce the widespread health problems of overweight, obesity and related co-morbidities among European consumers.

DiOGenes has integrated five strategic research, technological and development (RTD) lines, each with its specific objectives¹:

RTD1: Obesity and macronutrient composition of the diet

RTD2: Obesity and gene-nutrient interaction

RTD3: Obesity, genes & diet at the population level

RTD4: Obesity, consumer attitudes & behaviour

RTD5: Obesity and food technology

DiOGenes' aims are to provide innovative efforts that included all disciplines and stakeholders that could contribute to achieving a better understanding of how this disease can be prevented and treated from the dietary perspective.

The primary focus of DiOGenes was an investigation of dietary macronutrient components that will facilitate the prevention of weight gain and regain, studying specifically the role of the Glycemic Index (GI) of carbohydrates and of high dietary protein content for enhancing satiety, and thus reducing spontaneous energy intake. The objective has been to examine interactions between the dietary components and genetic and behavioural factors. DiOGenes provides a unique opportunity to identify gene-nutrient interactions associated with changes in body weight and waist circumference.

To examine critically the mechanistic impact of changes in macronutrient composition on weight control, a large long-term randomised, parallel dietary intervention study including whole families with both overweight, obese and normal weight members in 8 different countries across Europe has been conducted.

In addition, the intervention study has been coupled to analysis of genetic variation in candidate genes as well as novel approaches, such as gene expression in fat tissue and plasma peptidomics, gives the opportunity to identify sets of DNA polymorphisms, adipose tissue mRNA's and plasma peptides, ultimately enabling the prediction of an individual's response to nutrients in terms of weight change, which will in turn guide a diet-based treatment. Uniquely, the intervention study has been supported by detailed analysis of the psychological/behavioural responses of subjects, and with sophisticated measurement of free living energy expenditure by the doubly-labelled water technique. In the most comprehensive examination to date of the relation between diet and phenotype in the control of body weight, the consortium have aimed to identify key psychological/behavioural predictors of weight

gain given a genetic susceptibility, for use in diagnosing risk of weight gain and for better matching diets to consumer needs.

DiOGenes has major European food companies as partners. They are able to translate the newly developed knowledge into new food products, which will enable them to strengthen their competitiveness and benefit European consumers at risk from overweight or obesity.

Presentation of the dietary intervention study (RTD1)

This presentation will focus on RTD line 1: Obesity and macronutrient composition of the diet coordinated by Arne Astrup, University of Copenhagen, Denmark. The main objective of RTD1 was to identify the diet most effective for protection against weight gain and weight regain, in a susceptible population of obese and overweight individuals and their families, composed of spouse/partner and children.

The intervention study consisted of a 6 to 12 months dietary intervention study planned to enroll 450 families in 8 European centres, investigating the effect of different dietary components (high/normal protein and high/low glycaemic index carbohydrates) on maintenance of weight loss.

We chose to focus on GI and protein because a careful analysis of the literature pointed at GI and protein as the two most promising dietary factors for weight control with a potential for the food industry to produce optimised products taking new knowledge generated by the study into consideration.

The GI of carbohydrates has been demonstrated to have importance for cardiovascular risk factors and glycemic control in diabetics, and has been suggested to play a role in appetite control. A number of studies, and several popular diet books, attribute passive over-consumption of energy to a diet with a high GI, and suggest that replacing high GI foods with similar low GI foods can prevent this, or lead to a greater loss of fat compared to lean body tissue during dieting. The literature, however, is conflicting. Systematic reviews are equivocal and point to many negative studies that suffer from lack of dietary compliance and standardisation, and lack of statistical power to detect weight changes of clinical relevance and particularly of public health relevance. It is also pertinent to note that even the concept of GI has been questioned because of its poor reproducibility. Furthermore, most GI studies to date have been carried out with single sugars as opposed to real food. The current study has taken these shortcomings into consideration.

Ad libitum diets with high protein content have been shown to enhance satiety and increase thermogenesis in a number of smaller studies, and also produce better weight control and weight loss than diets in which the protein is replaced by carbohydrate. These high-protein diets are challenging the current recommendations and need to be evaluated for efficacy and safety in a large, long-term, strictly controlled intervention trial. A previous 12-month study found that a fat-reduced diet with high, compared to normal protein, appeared to enhance weight loss and provide a greater reduction in intra-abdominal fat stores after 6 months. However this pilot study did not have the statistical power to assess weight differences between groups after 12 months. The safety and tolerability of high protein diets in different age groups (particularly children) also requires clarification.

RTD line 1 have closely interacted with RTD lines 2, 3 and 4 to assess traits that determine the families' and individual family members' response to the tested diets. New concepts for food intake regulation generated in RTD line 5 will be used when composing the intervention diets.

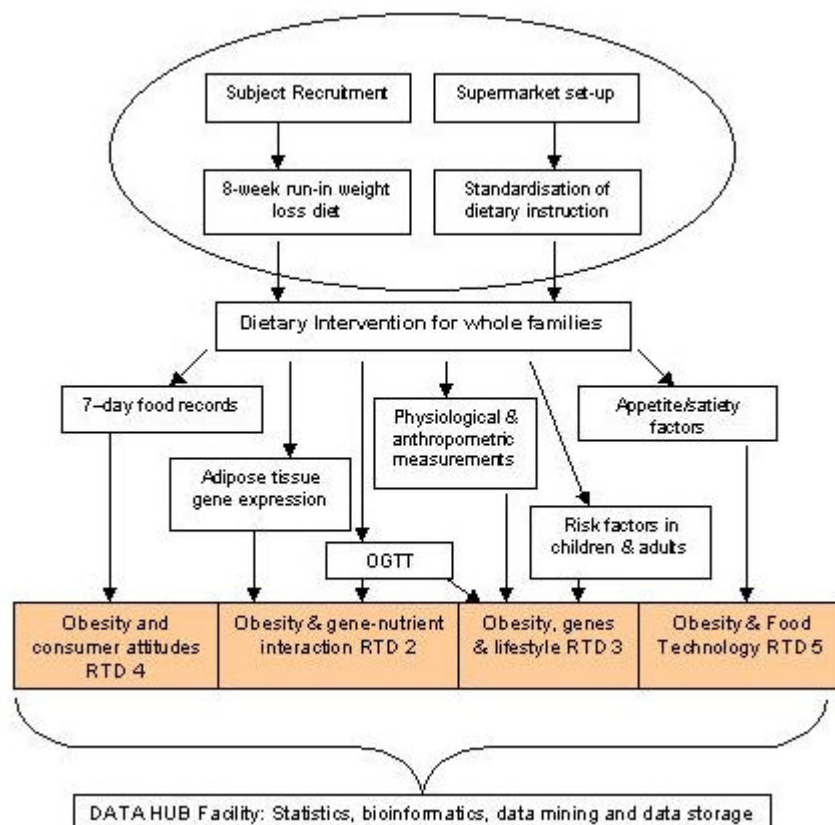
Dietary Intervention Study

The main objective of RTD1 will be achieved in a long-term 6 to 12 months dietary intervention study, using a novel factorial design to investigate the potential benefit of a high protein diet or a diet with low GI. The factorial design of the study (see below) has enabled a statistical assessment of any interactive effects of the different dietary arms. The study was conducted in 8 centres across Europe and was performed using a state of the art supermarket model in 2 centres for 6 months followed by 6 months of dietary instruction. The other 6 centres ran a 6-month study by instruction only. The objective was to assess responsiveness of the 450 families to different dietary compositions with putative effects on body weight control and risk factors during the dietary intervention study, with specific focus on changes in body weight and composition, appetite measures e.g. satiety (with RTD lines 4 and 5), adipose tissue gene expression (RTD line 2) and obesity related risk factors (RTD 3).

The potential risk associated with the dietary components of the intervention study with respect to CVD and diabetes type 2 in adults have been fully addressed during the

programme. Special attention was paid to minimising the burden for the children and to the potential risks associated with a high protein diet in children in particular, with respect to growth and metabolism.

A scheme of the RTD line 1 programme and interaction with other RTD lines is shown below:



The consortium aimed to recruit a total of around 850 obese/overweight parents (BMI>28) from the 8 participating centres, corresponding to 450 families with an estimated 450-1050 children, where at least one child in each family is overweight.

An initial 8-week run-in weight loss phase was a mandatory part for the overweight and obese parents only. To participate in the Diogenes study, adult family members were required to lose at least 8% of their body weight, before the whole family was admitted to the ‘dietary intervention’ part of the study and randomised to one of five diets. In two centres (Copenhagen and Maastricht) supermarkets set up for the study provided whole families with free food for 6 months, followed by 6 months of dietary advice and support. The other 6

centres (UK, Bulgaria, Crete, Czech Republic, Germany & Spain) provided families with 6 months of dietary advice and support only.

Thus the dietary intervention assessed weight re-gain after an initial weight loss. No energy restriction was imposed for the children in the enrolled families. The energy restricted Low Calorie Diet (LCD) consisted of 800-1000 kcal/d and the target macronutrient composition of the diet was 15-20% of total energy from fat, 35-40% from protein and 45-50% from carbohydrate. Families in which at least one of the overweight/obese subjects achieved the target weight loss ($\geq 8\%$ of initial body weight) were offered inclusion in the 6/12 months randomised weight maintenance (weight regain prevention) phase.

The children had to eat the same fat-reduced diet as the obese adults during the weight maintenance phase (cluster randomisation). The dietary intake was *ad libitum* (i.e. no restriction in total energy intake) and the intervention was based on the following 2x2 + 1 control factorial design:

	Low glycaemic index	High glycaemic index
Normal protein	1	2
High protein	3	4
Control	5	

1. 25-30% of energy from fat, 12% from protein and 55-60% from carbohydrates with a low GI.
2. 25-30% of energy from fat, 12% from protein and 55-60% from carbohydrates with a high GI.
3. 25-30% of energy from fat, 25% from protein and 45-50% from carbohydrates with a low GI.
4. 25-30% of energy from fat, 25% from protein and 45-50% from carbohydrates with a high GI.
5. Control diet, concordant with officially recommended diet.

Intervention via the supermarket model

The supermarket dietary intervention was conducted in 2 of the 8 centres, i.e. in Maastricht and Copenhagen. The system has been validated to allow a strict compliance to a targeted diet composition with concomitant allowance of variable energy intake depending on the satiating dietary effect etc. using the *ad libitum* principle. The food intake of the families is fully controlled, and subjects on the test diets got all their foods, including “empty calories”, from

the supermarket, which is like a normal supermarket with fresh, shelved and frozen products, split according to the dietary design. All food items are bar-coded and a dietician scanned the barcodes of all the chosen items to monitor macronutrient composition of the diet and assisted to alter the selection to meet the prescribed macronutrient composition. The calculated energy content of the chosen groceries was not made known to the subjects.

The control group was instructed to continue their habitual dietary intake whilst shopping in ordinary food shops. Subjects enrolled in the supermarket model in the 4 intervention groups received all foods free of charge during the first 6 months' intervention, and subsequently received careful, intensive and regular instructions during the second part of the intervention (from month 7 to 12). In the other 6 centres a similar intervention program was conducted, but with dietary instruction for 6 months only.

Intervention via dietary instruction (6 centres):

The centres not running the supermarket model conducted a dietary instruction every second week by a dietician. The intervention groups visited the dietician, who advised on weight control and reinforced the diet composition through recipes, cooking advice, and behaviour modification. The dietary instructions were standardized for all centres in terms of dietary content (protein type etc) but also reflected local dietary customs. Subjects were given oral and written instructions relating to the five intervention groups. The subjects in the four intervention groups participated in group-specific sessions every second week.

The primary outcome measures for adults were body weight loss maintained after the initial LCD weight loss, and weight regain. The secondary end-point were changes in body fat (kg), waist circumference, proportion of subjects maintaining ≥ 0 , ≥ 5 and $\geq 10\%$ weight loss, and drop-out rate. Main outcome measures for children were changes in the proportion of overweight and obese children at 6 and 12 mo, and changes in weight, height, waist-hip circumference ratio and body fat (%).

Secondary end-points also included reduction in abdominal obesity, changes in risk factors of type 2 diabetes and cardiovascular disease (CVD) and changes in physical activity. Blood samples were taken from adult subjects at baseline, after the 8-week run-in phase and after 6 and 12 months for analysis of risk factors and satiety factors.

Results

In the 8 centres taken all together 763 participating adults were successful in losing more than the requested 8%, they actually lost on average 11.2 kg during the 8 weeks low-calorie diet. After this successful weight loss phase, 565 families (763 adults and 787 children) were randomly distributed into 5 study groups, following different diets, either high or low in protein and/or high or low in glycemic index foods. A fifth diet, the control diet, was based on national healthy guidelines.

Results from the shop centres

As the complete analysis of results is pending, we here report only the results from the two supermarket centres². In these two centres the weight loss during the 8 week LED was 11±3 %. Subsequently, 263 adults (106 men, 157 women; initial body weight 99.8±15.6 kg, BMI 33.5±4.3 kg/m²) were randomized to the 5 *ad libitum* diets for 6 months: high protein/low GI (HP/LGI), HP/high GI (HP/HGI), low protein/high GI (LP/HGI), LP/low GI (LP/LGI) and control diet. 205 subjects (78%) completed the 6-month intervention. Their body weight had increased 1.5±4.6 kg or 16.0±40.9% of initial weight loss (P<0.001). Weight regain across diets was 2.1±3.9 (LP/LGI), 2.8±3.5 (LP/HGI), 0.8±4.3 (HP/LGI), 0.3±6.4 (HP/HGI), and 1.9±4.3 kg (control). Factorial ANOVA with BMI, gender and initial weight loss as covariates showed a significant main effect of protein content (P = 0.006 resp. 0.009), but no main effect of GI or interaction between protein and GI.

Conclusion:

The first results from the DioGenes dietary intervention study clearly show that an increased dietary protein content decreases weight regain after a weight loss, whereas the glycemic index did not play any detectable role. Consequently, we conclude that the efficacy of an increased protein content is important for prevention of weight regain, whereas a diet low in glycemic index possesses no advantage. The feasibility of these dietary changes in a more free setting is being assessed in the analysis of the corresponding outcomes in the 6 dietary instruction centres.

* DIOGENES is the acronym of the project ' Diet, Obesity and Genes' supported by the European Community (Contract no. FP6-513946), <http://www.diogenes-eu.org/>

References:

1. Saris WH, Harper A. DiOGenes: a multidisciplinary offensive focused on the obesity epidemic. *Obes Rev.* 2005;6:175-6.
2. M.A. van Baak, T.M. Larsen, S.A. Jebb, A. Kafatos, A. Pfeiffer, J.A. Martinez, S. Handjiev, M. Kunesova, A. Astrup, W.H.M. Saris. Weight loss maintenance on *ad libitum* diets varying in protein content and glycemic index: first results of the DIOGENES highly-controlled shop-based intervention. Hot topic abstract presented at the ECO 2008, Geneva.