Lipgene – Diet, genomics, and the metabolic syndrome: an integrated nutrition, agro-food, social and economic analysis






SIXTH FRAMEWORK PROGRAMME

Contents

Acknowledgment: This work was supported by *Lipgene*, an EU Sixth Framework Programme Integrated Project (Project number: FOOD-CT-2003-505944) (http://www.ucd.ie/Lipgene)

Foreword from Prof. Michael Gibney

The metabolic syndrome encompasses a series of conditions which are frequently seen together: abdominal obesity, hypertension, high blood lipids and insulin resistance. According to the World Health Organization, there will be 700 million adults with obesity by 2015. Clearly, prevention is the first option in combating the metabolic syndrome and, of course, curing the condition through sustained weight loss and physical activity is a second, but much less successful option. There is a third option which was the very centre of the *Lipgene* project: minimising some of the adverse effects through dietary change.

When the *Lipgene* consortium was being assembled back in 2003, limited human data and extensive data from animal studies suggested a strong effect of dietary fat levels and composition on one of the main elements of the metabolic syndrome, insulin resistance. Thus, one of the major tasks for *Lipgene* was to carry out the largest ever human intervention study of diet and the metabolic syndrome: 480 subjects followed for 12 weeks in 8 EU cities. The human genome sequence was released when *Lipgene* was being formed and we drew on a large French cohort of over 13,000 subjects who were followed for 8 years. We identified almost 877 of these who were healthy at the outset and remained healthy, and they were matched with an equal number of those who went on to develop the metabolic syndrome. This would allow us study how genes interact with nutrients to explain why some people do and others do not develop the metabolic syndrome.

Looking backwards into the food chain, we then asked the question: "can new technologies allow us to modify the fatty acids in the food chain?" We looked at new approaches to dairy and poultry nutrition and also how genetic engineering could enhance oil seed crops with algae genes to make long chain omega 3 fatty acids. But *Lipgene* also looked forward to consider possible outcomes and thus we studied the economics of changing the dietary fat supply and also the perception of consumers to the problem of the metabolic syndrome, to genetic testing to optimise nutrition and to their attitudes to new technologies.

I would like to thank all of those who worked on the *Lipgene* project and the staff of Directorate General Research for making this project so successful.

Prof. Michael Gibney
Principal Investigator
Institute of Food and Health
University College Dublin

Programme for the Final Conference

Held at the University College Dublin, Ireland on the 5th December 2008

0900 – 0915 **Registration, Coffee and Posters**

0915 – 0930 **Welcome and Introduction,** Prof. Christine Williams, University of Reading, UK

Opening of the Meeting, Dr. Patrick Cunningham,
Chief Government Scientific Advisor, Ireland

Chair: Prof. Mike Gibney, University College Dublin, Ireland

Session 1: Lipids, Genes and the Metabolic Syndrome

0930 – 1000 During this session, Prof. Helen Roche from University College Dublin, Ireland & Prof. Denis Lairon, INSERM France will outline the findings from *Lipgene* in relation to common genetic variations, how they interact with diet and predispose aspects of the metabolic syndrome.

1000 – 1010 *Questions*

Session 2: Optimising Fats in the Food Chain

1010 – 1030 Prof. Ian Givens from the University of Reading, UK will discuss efforts by *Lipgene* to use novel animal nutrition to modify the fat content of diet.

1030 – 1040 *Questions*

1040 – 1100 Dr. Thorsten Zank, BASF Plant Science, Germany will present the activities and outcome from *Lipgene* to use novel genetic methods to deliver a sustainable supply of polyunsaturated fatty acids from crop plants.

1100 – 1110 *Questions*

1110 – 1140 **Coffee, Posters and Prototype Tasting**

Session 3: What do European Consumers Think About the Metabolic Syndrome, Functional and Genetically Modified Foods?

1140 – 1200 Research from *Lipgene* assessing European opinion and attitudes towards genetic screening of people's diets (personalised nutrition) and the use of genetically modified foods will be presented by Prof. Maria Daniel Vaz de Almeida, University of Porto, Portugal and Dr. Barbara Stewart-Knox, University of Ulster, UK.

1200 – 1210 *Questions*

Session 4: Impact of the project

1210 – 1240 Prof. Ian Givens, University of Reading, UK will discuss how novel approaches to modify the fat content of foods can impact on cardiovascular health and the economic implications of such innovations in Europe.

1240 – 1250 *Questions*

1250 – 1305 **Panel Discussion**

1310 – 1315 **Conclusion and Close**

1315 – 1415 **Lunch and Posters**

Session 1: Lipids, Genes and the Metabolic Syndrome

Prof. Helen Roche, University College Dublin, Ireland

Speaker Biography

Helen Roche was recently appointed Associate Professor of Nutrigenomics at the Conway Institute, University College Dublin, Ireland. She is also a SFI Principal Investigator within the context of Molecular Nutrition. Prior to that as Wellcome Trust Fellow & Senior Lecturer in Molecular Nutrition at Trinity College Dublin, Dr. Roche established the first Nutrigenomics research group in Ireland, at the Institute of Molecular Medicine at Trinity College Dublin.

Prof. Roche has at least 50 publications cited in Medline. Recent work published in Diabetes (Impact Factor 9) presented novel findings with respect to inflammation in adipose tissue, insulin resistance and nutrient regulation of gene expression. Other top nutrition journals Prof. Roche's published work appears in include FASEB Journal, Journal of Immunology, American Journal of Clinical Nutrition & British Journal of Nutrition.

Prof. Denis Lairon, INSERM, France

Speaker Biography

Prof. Lairon completed a PhD in biochemistry in 1979. Following this he has undertaken roles including Research Director at Inserm (National Institute of Health and Medical Research) in 1999, Director of the Joint Research Unit 476 Inserm/1260 Inra/Université de la Méditerranée "Human Nutrition and lipids: bioavailability, metabolism and regulations" at Marseille, France from 1998-2007, and has been Vice-President (1994; 2005) and President (1995-98) of the French Nutrition Society. He is also president of the Federation of European Nutrition Societies (FENS), 2007-2011. As well as working on the *Lipgene* project, he is involved in two further European Projects, 3 COST actions, and NoE-NUGO.

Abstract

The metabolic syndrome is a very common disease associated with an increased risk of type 2 diabetes mellitus and cardiovascular disease. Diet and genetic susceptibility play a major role in the development and progression of the condition. In terms of dietary factors there is strong evidence to suggest that dietary fatty acid composition affects insulin sensitivity. Genetic background can interact with habitual dietary fat composition, thereby affecting predisposition to the metabolic syndrome and may also determine an individual's responsiveness to altered dietary fat intake.

Therefore *Lipgene* Human Nutrition Programme addressed this hypothesis in two ways. Firstly the *Lipgene* prospective case-control study determined the interaction between dietary fat composition and the development of the metabolic syndrome. Secondly the *Lipgene* Dietary Intervention Study determined the relative efficacy of reducing dietary saturated fatty acid (SFA) consumption, by altering quality and reducing the quantity of dietary fat, on metabolic risk factors of the metabolic syndrome.

The *Lipgene* prospective case-control study used the pre-existing SUVIMAX cohort (13,500 subjects followed 7y) to retrospectively select a *Lipgene* cohort (877 cases with metabolic syndrome and 877 matched controls). Genetic characteristics (182 candidate genes and 806 single nucleotide polymorphisms (SNPs)), baseline dietary composition focusing on fat intake and baseline plasma fatty acid composition (a biomarker of fat intake) were determined to study the interaction between dietary fat composition and genes associated with the risk of the metabolic syndrome in the development of the metabolic syndrome. As expected the phenotypes used to define the metabolic syndrome, including BMI, waist circumference, hypertension, elevated triacyl glyceride concentrations and low high-density lipoprotein levels were significantly different between metabolic syndrome cases and controls. Metabolic syndrome cases had significantly lower plasma total omega-6 and omega-3 polyunsaturated fatty acids (PUFA) but higher SFA (C14:0 & C16:0), compared to controls. Cases had lower education levels, more likely to be smokers and less physically active. Baseline dietary energy intake was higher in metabolic syndrome cases, whilst energy intake from fat was not different between groups. PCA analysis showed that plasma SFA and omega-6 PUFA were associated with increased metabolic syndrome risk, but long chain omega-3 PUFA were linked to reduced metabolic syndrome risk. Genetic markers linked with the development of the metabolic syndrome identified 7 single nucleotide polymorphisms in 6 genes, related to lipid metabolism and inflammation different between metabolic syndrome cases and controls. On-going analysis is defining interactions between apo B, C3 and other genes which are showing promising diet-gene interactions. In conclusion, this work showed that dietary fatty acids, and especially plasma fatty acids, are involved in the development of the metabolic syndrome while few gene polymorphisms can make subjects more or less susceptible.

In the *Lipgene* Human Intervention Study 417 free-living subjects with the metabolic syndrome received one of four dietary treatments for 12 weeks: (1) High-fat (38% energy) SFA-rich diet (HFSFA); (2) High-fat (38% energy), monounsaturated fatty acid (MUFA)-rich diet (HFMUFA); (3) Low-fat (28% energy), high-complex carbohydrate diet (LFHCC) and (4) Low-fat (28% energy), high-complex carbohydrate diet, with 1.24 g/d LC n-3 PUFA (LFn-3PUFA). Detailed metabolic (IVGTT, cytokines, adhesion molecules, coagulation factors and isoprostane analysis) with additional postprandial lipoprotein metabolism, skeletal muscle cellular fatty acid uptake and glucose disposal stable isotope studies and cellular mitochondrial status assessment pre- and post-intervention combined with genetic analysis revealed a vast amount of interesting data. Key results with respect to the effect of dietary fat modification on metabolic risk factors associated with the metabolic syndrome are presented here. Dietary fat modification had no significant effect on insulin sensitivity (SI) or insulin resistance (HOMA-IR) in the whole cohort. When habitual fat intake pre-intervention (high- or low-fat intake > or < the median 36% of energy from fat) was taken into account SI was significantly lower following the HSFA diet in subjects, particularly females, with a habitual low-fat intake pre-intervention. Insulin resistance (HOMA-IR) was reduced in females following the HMUFA diet. Plasma TAG, TRL and NEFA concentrations improved following the LFHCC omega-3 PUFA diet particularly in males. HDL-c concentrations were augmented by both high-fat diets. Dietary SFA modification had no effect on markers of inflammation, coagulation or oxidative stress. Postprandial lipid metabolism and skeletal muscle fatty acid handling was most affected by the LFHCC omega-3 PUFA diet. Interestingly inflammatory genes were linked to responsiveness to dietary therapy.

In conclusion therapeutic dietary therapy for the metabolic syndrome may require a 'personalised nutrition' approach, wherein habitual fat intake and genetic profile may determine responsiveness to specific dietary fatty acid interventions.

Session 2: Optimising Fats in the Food Chain

Prof. Ian Givens, University of Reading, UK

Speaker Biography

Prof. Ian Givens is currently Director of Animal Sciences Research Group (incorporating the Nutritional Sciences Research Unit, Biomathematics and the Centre for Dairy Research) in the University and joint leader of the University's Food Chain and Health Research Theme. He graduated from the University of Newcastle-upon-Tyne with a 1st class Honours Degree in Biochemistry and Nutrition with a subsequent PhD from the same university. Prof. Givens has worked for the Ministry of Agriculture, Fisheries and Food as a Nutritional Chemist and for ADAS as head of The Nutritional Sciences Research Unit. He has a particular interest in the relationship between the nutrition of animals, the composition of animal derived foods and their impact on human nutrition and chronic disease.

Abstract

In spite of the recognised benefits of reducing saturated fatty acid (SFA) intake few parts of the European Union (EU) meet recognised targets. Milk and dairy products represent the single largest source of dietary SFA in most countries, yet epidemiological evidence indicates that milk has cardioprotective properties such that simply reducing consumption of dairy foods to meet SFA targets may not be a sound public health approach. The animal nutrition workpackage has explored the options for replacing some of the SFA in milk fat with *cis*-monounsaturated fatty acids (MUFA) through alteration of the diet of the dairy cow and the evidence that such changes can improve the indicators for cardiovascular disease in general in the consumer. In addition the beneficial effects of long chain (LC) (carbon chain ≥20) omega-3 polyunsaturated fatty acids are well documented but recent evidence indicates that few people achieve the UK daily recommended intake for adults of 450 mg of EPA + DHA per day. In many parts of Europe the daily intake of EPA + DHA by adults and especially young people is less than 100 mg per day, since many never eat oily fish. Poultry meat contributes small but worthwhile amounts of EPA + DHA and studies to enrich the EPA + DHA content of animal-derived foods will be described and how this would impact on habitual intake.

Dr. Thorsten Zank, BASF Plant Science, Germany

Speaker Biography

Dr. Thorsten Zank studied Biology (Molecular Biology, Biochemistry & Plant Physiology) at the University of Hamburg, before going on to complete a PhD on the isolation and characterisation of genes involved in the biosynthesis of long chain polyunsatured fatty acids in the group of Prof. Dr. Ernst Heinz at the University of Hamburg in cooperation with BASF Plant Science (after 1 year post-doc). Since 2003 he has been working as a research scientist and innovation manager at BASF Plant Science, and has 10 years of expertise in the field of plant lipid biochemistry and molecular biology.

Abstract

Omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs) are widely accepted to have a positive effect on the prevention of diseases associated with the metabolic syndrome. The major source of these omega-3 LC-PUFAs is currently fish, while the primary producers of these omega-3 LC-PUFAs are marine microorganisms that form the basis of the marine food chain. However, since the demand for LC-PUFAs is increasing, fish stocks are decreasing drastically due to overfishing. Additionally, as a result of environmental pollution, fish is more and more enriched with harmful substances like heavy metals, dioxin and softeners.

It was therefore the goal of this joint effort between the University of York, Rothamsted Research and BASF Plant Science to develop an alternative, sustainable, safe and cheap source for LC-PUFAs for human nutrition. During the last five years, the partners aimed to modify the composition of vegetable oils—the traditional oil source for human consumption—towards substantial proportions of LC-PUFAs by novel genetic methods. The main advantages of LC-PUFAs produced in oilseed crops, such as rapeseed, compared to fish oil are (i) lower odour and sensory problems, (ii) less contamination in source oil, (iii) healthy vegetable fatty acids as by-products, in particular oleic acid, and (iv) plants as a sustainable and cheap source for fatty acids.

In a first step, genes have been identified from microorganisms (e.g. the alga *Thallasiosira pseudonana*) and transferred to rapeseed for seed specific expression. These transgenic plants have been used for detailed biochemical characterisation by applying various analytical tools developed during the course of the *Lipgene* project. These tools helped to get a comprehensive understanding of LC-PUFA biosynthesis within the seeds, and to identify bottlenecks limiting the accumulation of the desired LC-PUFAs to higher proportions. Several strategies aiming to circumvent these bottlenecks have been conducted, and the knowledge gained resulted in the successful genetic optimisation of oilseed rape towards the accumulation of significant proportion of LC-PUFAs in their seed oil.

Session 3: What do European Consumers Think About the Metabolic Syndrome, Functional and Genetically Modified Foods?

Prof. Maria Daniel Vaz de Almeida, University of Porto, Portugal

Speaker Biography

Professor Maria Daniel Vaz de Almeida, BSc (Human Nutrition), PhD (Nutrition), is a nutritionist, Professor of Public Health Nutrition / Community Nutrition at the Faculty of Nutrition and Food Sciences of Porto University. Currently, she is the national leader of several European funded Projects, namely: (1) Eating Out: Habits, Determinants, and Recommendations for Consumers and the European Catering Sector – HECTOR (2006-2009) (2) Promotion of vegetable and fruit consumption of school children – PRO GREENS (2008-2011) and (3) Expansion and update of existing nutrition monitoring systems – ANEMOS (2008-2010). In *Lipgene* she was the workpackage leader of the consumer science research. Together with Dr. Barbara Stewart-Knox she coordinated the qualitative and quantitative consumer research of the project. Her main research interests are determinants of food consumption, consumer attitudes and food intake.

Dr. Barbara Stewart-Knox, University of Ulster, UK

Speaker Biography

Barbara is currently a Senior Lecturer based within the Northern Ireland Centre for Food and Health (NICHE) at the University of Ulster, Coleraine. NICHE is an interdisciplinary research unit that received a five-star rating in the two previous research assessment exercises (RAE). Interests are broad within consumer health, diet and lifestyle. Principle investigator of several externally funded projects, Barbara has published 60+ papers in peer reviewed academic journals.

Abstract

Negative consumer opinion poses a potential barrier to the application of intervention to prevent and treat metabolic syndrome. These analyses have explored uptake and attitudes toward functional foods, GM foods and personalised nutrition among the European public. An omnibus opinion survey of a representative sample aged 14-55+ years (n=5967) took place in France, Italy, Great Britain (GB), Portugal, Poland and Germany during June 2005 as part of the *Lipgene* project. Individuals who are aware they have health problems associated with metabolic syndrome or who perceive themselves at risk of developing metabolic syndrome appear particularly favourable toward functional genetically modified (GM) food and nutrigenomic intervention. The findings are encouraging for the future uptake of GM food provided they deliver health and other tangible benefits and for the application of personalised nutrition provided that policies are put in place to address public concern about how genetic information is used and held.

Session 4: Impact of the Project

Prof. Ian Givens, University of Reading, UK

Speaker Biography

Prof. Ian Givens is currently Director of Animal Sciences Research Group (incorporating the Nutritional Sciences Research Unit, Biomathematics and the Centre for Dairy Research) in the University and joint leader of the University's Food Chain and Health Research Theme. He graduated from the University of Newcastle-upon-Tyne with a 1st class Honours Degree in Biochemistry and Nutrition with a subsequent PhD from the same university. Prof. Givens has worked for the Ministry of Agriculture, Fisheries and Food as a Nutritional Chemist and for ADAS as head of The Nutritional Sciences Research Unit. He has a particular interest in the relationship between the nutrition of animals, the composition of animal derived foods and their impact on human nutrition and chronic disease.

Abstract

In spite of the recognised benefits of reducing saturated fatty acid (SFA) intake few parts of the European Union (EU) meet recognised targets. Milk and dairy products represent the single largest source of dietary SFA in most countries yet epidemiological evidence indicates that milk has cardioprotective properties such that simply reducing consumption of dairy foods to meet SFA targets may not be a sound public health approach. This paper explores the impact of replacing some of the SFA in milk fat with *cis*-monounsaturated fatty acids (MUFA) on the on risk factors of cardiovascular disease (CVD) at population level. Results from a modelling exercise involving data for 11 EU Member States will be shown indicating that a worthwhile reduction in CVD would result from such changes. Moreover given the current and projected costs of health care, the results indicate that such changes would be cost-effective in a relatively short time. Modeling the impact of increasing the intake of long chain omega-3 on CVD at EU level of will also be briefly covered.

Posters from the *Lipgene* Consortium

The regulation of mTOR signalling by free fatty acids in human adipose tissue progenitors cells

Czech U, Balwierz A, Polus A, Kiec-Wilk B and Dembinska-Kiec A. *Department of Clinical Biochemistry Collegium Medicum, Jagiellonian University, Kraków, Poland.*

Development of (1) lipid-modified edible spreads and oils with omega-3 fatty acids and (2) promising communication routes for these lipid modified food prototypes

Wilma den Hoed, Ton van Immerseel, Chantalle Groeneschild, Liesbeth Zandstra, Maeve Cosgrove and Susan Vermunt. *Unilever Food & Health Research Institute, The Netherlands.*

Free fatty acids influence on aromatase gene expression in SVF cells

Wojciech Dudek, Łukasz Wator, Adriana Balwierz, Urszula Razny and Aldona Dembinska- Kiec. *Department of Clinical Biochemistry Jagiellonian University Medical College; Krakow, Poland.*

Gene-nutrient interactions in the metabolic syndrome: SNPs in ADIPOQ, ADIPOR1 and ADIPOR2 interact with plasma SFA levels to modulate insulin resistance in metabolic syndrome patients

Jane F Ferguson, Catherine Phillips, Audrey C Tierney, Jolene McMonagle, Pablo Pérez-Martínez, Catherine Defoort, Julie Lovegrove, Christian Drevon, Ellen Blaak, Aldona Dembinska-Kiec, Brita Karlström, Jose Lopez-Miranda and Helen M Roche. *Nutrigenomics Research Group, UCD Conway Institute, University College Dublin, Dublin.*

EPA and DHA Intakes in UK adults according to age, gender and income

Rachael. A. Gibbs and D. Ian Givens. *Nutritional Sciences Research Unit, School of Agriculture, Policy and Development, Faculty of Life Sciences, University of Reading, UK.*

LDAP expression in skeletal muscle biopsies from subjects with the metabolic syndrome

Ingrid MF Gjelstad, Fred Haugen, Kåre I Birkeland and Christian A Drevon. *Department of Nutrition, University of Oslo, Norway.*

Journey to LC-PUFA in plants: moving from models to crops

Richard P Haslam, Monica Venegas-Caleron, Olga Sayanova, Johnathan A Napier, Tony R Larson, Ian A Graham, Thorsten Zank, and Joerg Bauer. *CNAP, Department of Biology, University of York, UK.*

Metabolic syndrome & psychosocial factors

Stephanie Hodge and Barbara Stewart-Knox. *Northern Ireland Centre for Food & Health University of Ulster, Coleraine, UK.*

Effect of normal and high oleic rapeseed in the dairy cow diet on milk fatty acid composition

Kirsty E. Kliem, David J. Humphries and D. Ian Givens. *Animal Science Research Group, School of Agriculture, Policy and Development, University of Reading, UK.*

Estimation of current and projected omega-3 fatty acid intake with enriched food prototypes in The Netherlands, United Kingdom, and France

Kati M Laitinen, Jennifer Eeuwijk, Susan HF Vermunt and Peter L Zock. *Unilever Food & Health Research Institute, The Netherlands.*

Hyphenated-Chromatography techniques to monitor the oilseed metabolome during LC-PUFA synthesis

Tony R. Larson, Valeria Gazda, Johnathan A. Napier, Thorsten Zank and Ian A. Graham. *CNAP, Department of Biology, University of York, UK.*

Dietary habits and metabolic syndrome in middle-aged europeans

Heather Parr, Barbara Stewart-Knox, Bunting B, Gilpin S, Pinhão S and de Almeida MDV. *Northern Ireland Centre for Food and Health, University of Ulser, Coleraine*

Complement component 3 polymorphisms and the metabolic syndrome: Interaction with polyunsaturated fatty acids modulates the risks

Catherine M Phillips, Louisa Goumidi, Sandrine Bertrais, Jane F Ferguson, Ross McManus, Serge Hercberg, Denis Lairon, Richard Planells and Helen M Roche. *Nutrigenomics Research Group, School of Public Health and Population Science, Conway Institute, University College Dublin, Ireland.*

Influence of free fatty acids on the the expression of adiponectin during differentiation of the human adipose tissue SVF cells

Urszula Razny, Urszula Czech, Adriana Balwierz and Aldona Dembinska-Kiec. *Department of Clinical Biochemistry Jagiellonian University Medical College, Cracow, Poland.*

N-3 polyunsaturated fatty acid effects on the cardio-metabolic risk in rats.

Delphine Rousseau, Vanessa Robbez Masson and Alain Grynberg. *INRA – UMR-A 1154 Lipides membranaires et Régulation fonctionnelle du Cœur et des Vaisseaux – Faculté de Pharmacie, IFR 141, Université Paris-Sud XI, France.*

Enriching poultry meat with long chain n-3 fatty acids

C. Rymer, R.A. Gibbs and D.I. Givens. *Animal and Nutritional Sciences Research Unit, School of Agriculture, Policy and Development, University of Reading, UK.*

Effects of eicosapentaenoic acid on fatty acid oxidation and metabolic flexibility in human skeletal muscle cells

AC Rustan, A Fjørkenstad, NP Hessvik and GH Thoresen. *Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo, Norway.*

Individual fatty acids and inflammatory gene expression in endothelial cells: impact of cell stimulation state

DI Shaw, NR Jeffs, WL Hall and CM Williams. *Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences, University of Reading School of Food Biosciences, UK*

Effects of dietary fat modifications on insulin sensitivity and metabolic markers of the metabolic syndrome. *Lipgene* – a randomised European dietary intervention study

Audrey C Tierney, Jolene McMonagle, Danielle I Shaw, Hanne L Gulseth, Catherine Defoort, Wim H.M Saris, Juan A Paniagua, Iwona Gołąbek-Leszczyńska, Denis Larion, Christine M Williams, Brita Karsltröm, Bengt Vessby, Aldona Dembinska-Kiec, José López Miranda, Ellen Blaak, Christian A Drevon, Michael J Gibney, Julie A Lovegrove and Helen M Roche. *Nutrigenomics Research Group, UCD Conway Institute, University College Dublin, Republic of Ireland.*

Effect of dietary fat modification on skeletal muscle fatty acid handling in the metabolic syndrome; a stable isotope approach

Anneke MJ van Hees, Wim HM Saris, Gabby B Hul, Nicolaas C Schaper, Bas E Timmerman, Helen M Roche and Ellen E Blaak. *Department of Human Biology (NUTRIM), Maastricht University, The Netherlands.*

Relating milk composition to the microbial ecology of the rumen

John Wallace and Graham Horgan. *University of Aberdeen Rowett Institute for Nutrition and Health, UK*

Dietary supplementation of tetradecylthioacetic acid increases feed intake, but reduces body weight gain and adipose depot sizes in rats fed high-fat diets

Andreas J. Wensaasa, Arild C. Rustanb, Merethe H. Rokling-Andersena, Jørgen Jensenc, Olav Kaalhusd, Bjørn A. Graffe, Oddrun A. Gudbrandsenf, Rolf K. Berge, and Christian A. Drevon. *Department of Nutrition, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Norway.*

The regulation of mTOR signalling by free fatty acids in human adipose tissue progenitors cells

Urszula Czech, Beata Kieć-Wilk, Adriana Balwierz, Anna Polus and Aldona Dembińska-Kieć.
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Introduction
Mammalian target of rapamycin (mTOR) has emerged as a regulator of development and growth related processes like response to hypoxia, energy status and ageing mTOR plays a major role in signaling caused by nutrients independently to mitogens such as growth factors. TOR pathway up-regulation (excess of energy) promotes fat accumulation and insulin resistancy, thus plays an important role in development of metabolic syndrome and type 2 diabetes. mTOR regulation is also involved in angiogenesis by activation of expression of HIF-1 dependent genes.

Methods
The modified Hauner's method was used to obtain the human subcutaneous adipose tissue progenitor-stromal vascular fraction (SVF) cells. SVF was incubated with arachidonic acid (AA), palmitic acid (PA), eicosapentaenoic acid (EPA) and oleic acid (OA) in the concentrations- 10μM (AA) and 30μM (PA, EPA, OA) for 24h. Total RNA was isolated using Trizol (Sigma) and SV total RNA isolation system (Promega). The high grade purity RNA was used for oligonucleotide microarray (HG-U133 A, Affymetrix, 14 500 genes). The influence of FFA on gene expression was measured by real-time PCR method. To measure the activity of kinases-AKT, FAK, p38 and ERK1/2- Fast Activated Cell-based ELISA (FACE™) Kits were used.

Results
FFA regulate the mTOR pathway in SVF: Rheb was upregulated by unsaturated fatty acids, especially by EPA. It was paralelled by activation of AKT. The analysis of microarrays data suggests that fatty acids stimulate differentiation of SVF into adipocytes.

All used fatty acids induced the activation of AKT kinase (inhibition of apoptosis). The reduced activity of FAK kinase was observed after stimulation with fatty acids, what may be connected with reduction of cell- matrix adhesion (confirmed by down regulation of adhesion molecule genes found in microarray). The p38 kinase activity was down-regulated by unsaturated fatty acids in SVF cells. Changes in activity of ERK kinase was not observed, what is in agreement with the lack of stimulation of cell proliferation by FAs.

Conclusions
FFA regulated the upstream pathway of mTOR (the TSC1–TSC2 complex and Rheb) by which TOR may influence the cell cycle apoptosis and differentiation of SVF cells by AMPK and AKT-induced fosforylation.

Acknowledgements: This work was supported by *Lipgene*, an EU Sixth Framework Programme Integrated Project (Project number: FOOD-CT-2003-505944) and Polish MNiI project Nr 2 P05 132 28

Development of (1) lipid-modified edible spreads and oils with omega-3 fatty acids and (2) promising communication routes for these lipid modified food prototypes

Wilma den Hoed, Ton van Immerseel, Chantalle Groeneschild, Liesbeth Zandstra, Maeve Cosgrove and Susan Vermunt. *Unilever Food & Health Research Institute, The Netherlands*.
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Introduction

The demonstration workpackage started in April 2005. The key objectives were to develop lipid-modified edible food prototypes that can be substituted in the diet with 1) health benefits relevant to the metabolic syndrome (MS); and 2) acceptable sensory and physical properties; and to 3) develop promising communication routes for these lipid modified food prototypes.

Materials and Methods

Three manufactured prototype spreads and salad oils were developed to be enriched with fish oils or linseed & rapeseed oils. The spreads were tested in a reversed shelf-life R-index test by an experienced sensory panel (n=9). In addition, 1502 participants from Germany, Italy and Portugal completed an online consumer test where consumers chose their preferred concepts in relation to pictures of lipid modified poultry meat, spreads or cheese in Germany, Italy and Portugal.

Results

A stable low fat (25%) spread with 2g ALA/20g serving; a low fat (25%) spread with 500mg EPA & DHA/20g serving and a salad oil with 2g ALA and 500mg EPA & DHA/14g serving were produced with no significant sensory differences between prototype and fresh reference product. Different communication concepts affected how participants judged the products; overall the 'reason to believe' influenced most the preference of health statements.

Conclusion

Spreads and oils enriched with omega-3 fatty acids were stable up to 12 and 19 weeks respectively; any differences in sensory attributes were small and expected not to be perceived by consumers. In terms of communication around MS food prototypes, consumers preferred negative framing i.e. 'the unhealthy situation that can be avoided' above positive framing i.e. "the positive situation that can be reached'.

Funding

This work was supported by *Lipgene*, an EU Sixth Framework Programme Integrated Project (Project number: FOOD-CT-2003-505944) and Unilever.

Free fatty acids influence on aromatase gene expression in SVF cells

Free fatty acids influence on aromatase gene expression in SVF cells

Wojciech Dudek, Łukasz Wator, Adriana Balwierz, Urszula Razny and Aldona Dembinska- Kiec.
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Introduction: Adipose tissue is known as a secretive non only energy storing organ. Aromatase is one of the substances produced by adipose tissue. This enzyme is also expressed in in gonads, vessel wall cells. In adipose tissue the enzyme is expressed by stromal vascular fraction, not adipocytes. It is the main enzyme for estrogen syntase which drives androgens as androstenedione, testosterone and 16α-hydroxyandrostenedione to estrogen convertion.

AIM of the study was to investigate influence of fatty acids on expression of aromatase gene in progenitor cells isolated from human adipose tissue – stromal vascular fraction cells SVF.

Materials and Methods: SVF cells were isolated using modified Hauner's method. After adaptation (5-7 days) in Adaptation Medium (SVF-AM, DMEM + insulin + hydrocortisone + transferin) cells were stimulated to differentiate to adipocytes (SVF-Adipo, by 48h incubation with adipogenic factors: IBMX, insulin, dexamethasone and culturing in Adipo medium- (DMEM + insulin + hydrocortisone + transferin + triiodothyronine) or to endothelial cells (SVF-Angio, by 24h incubation in Angio medium- EBM+2%FCS). Then cells (SVF-AM, SVF-Adipo, SVF-Angio) were for 24 h incubated with non-toxic (10-30 μM) concentrations of fatty acids (FFA): arachidonic acid-AA, eicosapentaenoic acid-EPA, oleic acid-OA and palmitic acid-PA). After incubation with FFA, the relative aromatase gene expression was performed using real-time PCR.

Results: OA upregulated aromatase gene expression while AA and EPA downregulatted it in AM medium (not differentiated SVF cells). In cells differentiated to adipocytes (Adipo medium) after incubation with fatty acids no differences in aromatase gene expression were observed, whereas in cells differentiated to angioblasts (Angio medium) PA and OA downregulated aromatase gene expression while AA and EPA upregulatted it.

Conclusion: Fatty acids could differently regulate aromatase gene expression in SVF cells what depends on the type of fatty acid (poly- and mono- unsaturated) and the stage as well as direction of cell differentiation.

Acknowledgements: This work was supported by *Lipgene*, an EU Sixth Framework Programme Integrated Project (Project number: FOOD-CT-2003-505944) and Polish MNiI (N404 008 32/0237) project.

Gene-nutrient interactions in the metabolic syndrome: SNPs in ADIPOQ, ADIPOR1 and ADIPOR2 interact with plasma SFA levels to modulate insulin resistance in metabolic syndrome patients

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Introduction/objectives
Progression of the metabolic syndrome is determined by genetic and environmental effects. Gene-nutrient interactions may be important in modulating susceptibility to development of metabolic syndrome traits. Here, gene-nutrient interactions were examined in subjects with the metabolic syndrome to determine interactions between single nucleotide polymorphisms (SNPs) in the adiponectin gene (ADIPOQ) and its two receptors (ADIPOR1 and ADIPOR2), and plasma fatty acid composition on characteristics of the MetS.

Materials and methods/aims
The *Lipgene* human intervention study determined the effect of reducing dietary saturated fatty acid (SFA) intake, by replacement with monounsaturated fatty acids (MUFA) or as part of a low-fat (LF) diet for 12 weeks, to alter plasma fatty acid composition in 486 subjects with the MetS. Plasma fatty acid composition, insulin sensitivity as assessed by IVGTT, biomarkers of inflammation, and ADIPOQ, ADIPOR1 and ADIPOR2 SNP genotypes were determined.

Results
In the whole cohort, four SNPs interacted with plasma SFA to significantly associate with insulin and HOMA, while two SNPs interacted with plasma SFA to affect adiponectin levels. However, gender-specific analysis revealed that the association with insulin sensitivity was stronger in males than in females. The gene-nutrient interaction was strengthened by SNP combination into multi-locus genotypes, revealing sub-groups of individuals with modifiable insulin resistance phenotypes, whose insulin and HOMA changed significantly over the intervention period depending on changes in plasma SFA.

Conclusions
Polymorphisms in ADIPOQ, ADIPOR1 and ADIPOR2 showed consistent interactions with plasma SFA which may modulate insulin sensitivity in the MetS. Gender may be an important factor in determining the strength of the effect.

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EPA and DHA intakes in UK adults according to age, gender and income

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Introduction

In a study of UK adults, mean intake of eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) was 244mg/d which was sub-optimal relative to targets[1]. Variation due to age, gender or socioeconomic differences remains unknown. Therefore the objective of this work was to examine trends in intakes of EPA+DHA in males and females according to age group in the main UK population and a low income subgroup.

Materials and Methods

Diet diary data from the National Diet and Nutrition Survey[2] and Low Income Diet and Nutrition Survey[3] were used to calculate intakes of fish (exc.canned tuna) and animal derived foods by age and gender. These data, coupled with values for fatty acid composition of each food type gave estimates of EPA+DHA intakes.

Results

EPA+DHA intakes for adults aged 19-24, 25-34, 35-49 and 50-64 years in the national population were 97, 172, 249 and 334mg/d respectively for males and 98, 136, 203 and 328mg/d respectively for females. Overall mean intake for males was 259mg/d and 226mg/d for females. In the LIDNS subgroup, overall mean EPA+DHA intake was 183mg/d for males and 199mg/d for females and according to age group, intakes for 19-34, 35-49, 50-64 and 65+ years were 134, 133, 247 and 209mg/d respectively for males and 127, 233, 212 and 204mg/d respectively for females.

Conclusions

A trend towards increasing intakes with age and modestly higher intakes in males than females is apparent in both groups. Intakes in the low income group are lower, although differing survey methods and age groupings do not allow full comparison. If eating habits do not change, today's younger adults are likely to have vastly suboptimal intakes in the future.

Acknowledgements

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[3] Nelson *et al* (2007) LIDNS.

LDAP expression in skeletal muscle biopsies from subjects with the metabolic syndrome

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Background
Lipid droplet associated proteins (LDAPs) are found on the surface of intracellular lipid droplets in adipose tissue, skeletal muscles as well as other tissues. Five LDAPs have been described: Perlipin (PLIN), Adipofilin (ADFP), TIP47 (M6PRBP1), S3-12 (KIAA1881) and OxPAT (LSDP5). Some of these proteins are necessary for triglyceride accumulation and hydrolysis in cells, and the gene expression can be regulated by fatty acids as well as other conditions. Increased amounts of intramuscular fat has been observed both in obese and trained, normal weight subjects. In obese subjects intramuscular lipid accumulation is associated with reduced insulin sensitivity. This work set out to determine whether there is any relationship between mRNA expression of LDAPs in skeletal muscle biopsies and: quantity and quality of dietary fat; body weight; physical activity level (PAL); and insulin sensitivity.

Material and Methods
mRNA expression of five LDAPs was monitored in skeletal muscle biopsies (m. vastus lateralis) from 30 men and 32 women with the metabolic syndrome from Oslo, Maastricht, Uppsala and Dublin. The biopsies were taken before and after 3 months in the *Lipgene* human dietary intervention study (WP 1.2). Gene expression was analyzed by TaqMan Gene Expression Assays (RT-PCR, Applied Biosystems) using RPLP0 as endogenous control. Dietary intake was calculated from 3 days weighed food records before and after the intervention. PAL was calculated based on the Baecke questionnaire filled in at baseline.

Preliminary Results
At baseline there was a negative correlation between dietary intake of saturated fatty acids (%) and two LDAPs in men, but not in women (LSDP5: $r=-0.58$, $p=0.001$ and ADFP: $r=-0.49$, $p=0.006$). There was no correlation between total fat intake (%) and any of the LDAPs. A positive correlation existed between body weight (kg) and PLIN in men ($r=0.57$, $p=0.001$) as well as women ($r=0.41$, $p=0.020$). There was a negative correlation between PAL (arbitrary units, AU) and both LSDP5 ($r=-0.72$, $p<0.001$) and KIAA1881 ($r=-47$, $p=0.017$) in women, but not in men. There was also a negative correlation between fasting C-peptide and ADFP in men ($r=-0.43$, $p=0.017$), but it did not reach statistical significance in women ($r=-0.34$, $p=0.061$). The correlation between fasting insulin and glucose with ADFP was not significant, and there was no change in LDAP expression after 3 months of dietary intervention.

Conclusions
Preliminary baseline results show sex specific correlations between mRNA expressions of LDAPs in skeletal muscle biopsies, some dietary and biological parameters as well as physical activity level. mRNA levels of LDAPs did not change after 3 months of dietary intervention focusing on quality and quantity of dietary fatty acids.

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Journey to LC-PUFA in plants: moving from models to crops

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Humans are capable of only limited *de novo* synthesis of long chain polyunsaturated fatty acids (LC-PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Therefore the human diet must contain these essential fatty acids. In particular EPA and DHA derived from fish have been shown to improve human health. In particular it is suggested that *n-3* LC-PUFA may reduce the risk of metabolic syndrome, a term used to describe a collection of pathologies indicative of a progression towards heart disease, diabetes, stroke and obesity, which constitutes an increasing public health problem in industrialised societies. The ability to produce such fatty acids in plants, as an alternative to marine sources, is therefore of considerable interest.

Production of such fatty acids requires a biotechnological multidisciplinary approach in which a suite of genes, including novel desaturases, elongases and acyltransferases are introduced into plants in order to modify the oil. A process of gene discovery from a wide variety of organisms e.g. diatoms and fungi, has provided a portfolio of candidate genes with optimal substrate preferences and activities. Genes encoding these activities have been combined in cassettes and introduced into the model plant Arabidopsis. With LC-PUFA biosynthesis demonstrated at significant levels in model plants, work then focused on the delivery of oils in an appropriate agricultural crop, *Brassica napus* or rapeseed. A combination of glass house and field trials has confirmed the utility of producing LC-PUFA in *B. napus*. We have demonstrated through this work how the modification of plant oils can be achieved in crops of agricultural significance.

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Metabolic syndrome & psychosocial factors

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Socio-economic and lifestyle factors are known to influence development of the metabolic syndrome and there is some evidence to suggest that stress and other negative psychosocial factors may also contribute. There is however very little evidence as to whether positive emotional factors, such as resilience or optimism, may or may not reduce the risk of developing metabolic syndrome. The aim of this study has been to explore potential associations between various positive and negative psychological, lifestyle and social factors in people with metabolic syndrome compared to healthy individuals. Data were collected by self-reported questionnaire from people aged 30 to 70 (n=200) previously screened for the *Lipgene* nutritional studies being carried out in Ireland (ROI), UK, and France (n=110) and also by direct approach a representative sample of the general public (n=90). Data were analysed using SPSS Version11.5 for Windows. Results are shown for psychosocial and lifestyle factors, and are discussed in relation to identification of groups and areas for targeting of health promotion to prevent and treat metabolic syndrome.

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Effect of normal and high oleic rapeseed in the dairy cow diet on milk fatty acid composition

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Introduction

Milk and dairy products are the major source of saturated fatty acids (SFA) in the European diet and there is evidence that replacing SFA with *cis*-monounsaturated fatty acids (MUFA) will improve risk factors for cardiovascular disease. Rapeseed in dairy cow diets simultaneously reduces milk SFA and increases *cis*-MUFA, but also increases milk *trans* MUFA. A new variety of rapeseed with higher oleic acid content (therefore less potential for increasing milk *trans*-MUFA) was compared with a normal oleic acid rapeseed, and both compared with a control diet.

Methods

Seven early lactation multiparous Holstein-Freisian cows were randomly allocated to one of seven total mixed ration diets (50:50 forage:concentrate) for 5 x 28 day periods. The control diet contained 41 g/kg dry matter commercial lipid supplement and treatment diets contained one of two different rapeseeds (normal and high, oleic acid contents 58 and 70 g/100 g total fatty acids respectively) milled with wheat at three inclusion levels so that diets provided approximate intakes of 750, 1000 and 1250 g/cow/day rapeseed oil. Milk fatty acid composition was analysed at the end of each period.

Results

Both rapeseeds decreased ($P<0.001$) milk SFA content from 66 g/100g fatty acids in control milk to 58 and 57 g/100g fatty acids for the highest dose of normal and high oleic rapeseed respectively. Corresponding increases in *cis*-MUFA ($P<0.05$) were seen (from 25 to 31 and 33 g/100g fatty acids). These responses were linear, and greater for the high oleic acid rapeseed. Milk *trans*-MUFA content was also increased by feeding both rapeseeds ($P<0.05$) but there was no difference between rapeseed types.

Conclusions

The results indicate that the high oleic acid rapeseed was superior to the normal at decreasing and increasing SFA and *cis*-MUFA respectively but this was still accompanied by increases in milk *trans*-MUFA.

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Estimation of current and projected omega-3 fatty acid intake with enriched food prototypes in The Netherlands, United Kingdom, and France

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Introduction

A significant part of the European population may not have intakes of omega-3 fatty acids at the level recommended for prevention of cardiovascular disease. This study provides an overview of the population average intakes of α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in The Netherlands, United Kingdom (UK) and France. Another objective was to investigate the potential impact of enriched food prototypes on the population average intake of these fatty acids.

Materials and Methods

The average intakes of ALA, EPA and DHA in the general healthy adult population were estimated by combining food intake data from national dietary surveys of each country with the fatty acid composition of foods as reported in respective national food composition tables. To estimate the potential impact of prototype foods (fat spreads and salad oils enriched with ALA; and poultry meat, fat spreads and salad oils enriched with EPA and DHA) on the intake of ALA and EPA and DHA it was assumed that prototypes would replace all equivalent regular foods.

Results

The estimated average intake of ALA was 1.6, 1.1 and 0.8g/d in the Netherlands, UK and France, respectively. The estimated average intake of EPA and DHA was 75, 246 and 226mg/d in the Netherlands, UK and France, respectively. With prototypes the potential intake of ALA was predicted to be 2.7, 1.8 and 0.9 g/d in The Netherlands, UK and France, respectively. Similarly the potential intake of EPA and DHA was predicted to be 461, 512 and 327 mg/d in The Netherlands, UK and France, respectively.

Conclusion:

The average intake of ALA, EPA and DHA is lower than recommended for the prevention of cardiovascular diseases. The availability of common, everyday foods enriched with ALA, EPA and DHA could help people achieve optimal omega-3 fatty acids intake levels.

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Hyphenated-Chromatography techniques to monitor the oilseed metabolome during LC-PUFA synthesis

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Introduction

Crop species such as oilseed rape do not accumulate long-chain polyunsaturated fatty acids (LC-PUFAs) in their seed triacylglycerols (TAGs). LC-PUFAs such as eicosapentaenoic acid (EPA) can be produced if novel desaturases and elongases are identified and expressed in developing seeds[1]. The success of this approach requires the iterative integration and co-ordinate expression of these genes to reconstitute the LC-PUFA pathway. To inform the success of this and other approaches we have developed a platform of technologies to discover the bottlenecks in LC-PUFA production in metabolically engineered oilseeds.

Materials and Methods

A *Brassica napus* line expressing several desaturases and elongases from heterologous sources was used to evaluate LC-PUFA pathway activity in developing seeds. In addition, an *Arabidopsis thaliana* LC-PUFA line producing low levels of EPA was subjected to large-scale mutagenesis to introduce genetic variation and subsequently screened for enhanced EPA production. Seed material was profiled using hyphenated chromatography platforms developed for TAG, acyl CoA, and fatty acid methyl ester (FAME) analyses.

Results

Analyses of *B.napus* seed showed that LC-PUFAs generally accumulated on only one of the three available TAG positions. This suggests that acyltransferase engineering in addition to desaturase/elongase expression is required to maximize LC-PUFA production. The accumulation of acyl-CoA intermediates, specifically delta-6 elongase substrates, also suggested that this elongation step was sub-optimal. Finally, FAME screening of the mutagenized *A thaliana* population showed that further increases in LC-PUFA yield are possible.

Conclusions: Metabolite profiling platforms have proved invaluable to guide the process of LC-PUFA metabolic engineering in oilseeds. Candidate lines can be evaluated not only in terms of LC-PUFA yield, but also to discover what specific biochemical steps require further optimization.

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Dietary habits and metabolic syndrome in middle-aged europeans

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Metabolic syndrome may be dietary related[1]. The aim of this analysis has been to explore associations between dietary habits, body fat distribution and patterns in the reporting of conditions related to metabolic syndrome in European consumers. Data were collected by survey from a representative sample aged ≥40 years (*n* 1722) in GB (*n* 1182) and Portugal (*n* 540) as part of the *Lipgene* project. Waist circumference (WC) and BMI were measured. A short Food Frequency Questionnaire (FFQ) was employed to assess dietary habits for major food groups. FFQ data were factor analysed, and self-reported conditions associated with metabolic syndrome (high blood cholesterol, high blood pressure, mid-waist obesity, high/low blood sugar) and anthropometric measures underwent Latent Class Analysis M*plus*©[2].

Based on FFQ factor analysis three clusters of dietary habits emerged: 'alcohol rich' with infrequent intake of fruit and vegetables or other food groups; 'unhealthy' frequent consumption of high-fat-containing foods and infrequent alcohol; and, 'healthy' with wine consumption (infrequent intake of other alcohol) and consumption of a variety of food groups including fish and low-fat-containing foods. Four latent classes emerged from patterns of self-reported metabolic syndrome signs ('metabolic syndrome' (22%), BMI- 29.9, WC- 97.9cm, 'healthy' (no symptoms) (58.4%) BMI- 24.9 WC- 84.8cm; 'overweight' (15%), BMI- 28.4, WC- 92.5cm; 'obese' (4.6%), BMI- 35.9, WC- 102.2cm). One way ANOVAs were conducted to determine differences between four metabolic syndrome latent classes in relation to three dietary profiles. The 'metabolic syndrome' class were less likely than the 'overweight' class to be in either the 'unhealthy' ($p=0.000$) or 'alcohol rich' ($p=0.023$) dietary group and the 'metabolic syndrome' class were less likely than the 'healthy' class to be in the 'unhealthy' ($p=0.000$) or 'alcohol rich' ($p=0.007$) dietary groups. The findings could suggest that those reporting signs of metabolic syndrome are not defined by unhealthy dietary habits. Frequent alcohol consumption appears to be a marker for infrequent food consumption and infrequent alcohol a marker for intake of high-fat foods, while wine intake may be a marker for a healthy balanced diet. This theory requires further investigation.

This work was supported by *Lipgene*, an EU Sixth Framework Programme Integrated Project (Project number: FOOD-CT-2003-505944).

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Complement component 3 polymorphisms and the metabolic syndrome: Interaction with polyunsaturated fatty acids modulates the risks

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Objectives
Complement component 3 (C3) is a strong determinant of the metabolic syndrome. Dietary fat is a key environmental factor which may interact with genotype to affect metabolic syndrome risk. We investigated the relationship between C3 polymorphisms and the metabolic syndrome, and determined their modulation by plasma fatty acids, biomarkers of dietary fat intake.

Research Design and Methods
C3 polymorphisms, biochemical measurements and plasma fatty acids were determined in the *Lipgene* WP1.1 cohort of metabolic syndrome cases and matched controls (n=1754).

Results
C3 and CRP were higher in the metabolic syndrome cases (P<0.0001) and displayed a significant dose relationship with the number of metabolic syndrome components (P=0.000). For rs2250656, AA homozygotes had higher metabolic syndrome risk compared to the GG homozygotes (OR=2.76, CI 1.23-6.18, P=0.001) and G allele carriers (OR=1.74, CI 1.13-2.67, P=0.01), which was exacerbated by low polyunsaturated fatty acid (PUFA) levels (OR 2.24, CI 1.04-4.81, P=0.04). A allele carriers had higher C3 levels (1.52±0.02 vs 1.37±0.05 P=0.005) which were modulated by n-3 PUFA levels (P=0.04). A allele carriers also had greater abdominal obesity (P=0.006), higher CRP, triglyceride (TAG) and lower HDL levels, and were less insulin sensitive (P<0.05). For rs11569562, GG homozygotes had reduced metabolic syndrome risk (OR 0.52, CI 0.32-0.83, P=0.007). High PUFA levels ameliorated this protective effect (OR for metabolic syndrome risk 0.30, CI 0.09-0.92, P=0.04). GG homozygotes had lower C3 and CRP levels compared to the AA homozygotes (1.48±0.03 vs 1.58±0.03 P=0.03 and 1.92±0.17 vs 2.56±0.27 P=0.02) and they also had reduced risk of high TAG levels compared to the A allele carriers (OR 0.52, CI 0.30-0.90, P=0.02) which were modulated by long chain omega-3 PUFA s (P=0.01).

Conclusions
C3 polymorphisms influence the risk of the MetS and its phenotypes. Gene-nutrient interactions with plasma PUFAs modulate these effects.

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Influence of free fatty acids on the the expression of adiponectin during differentiation of the human adipose tissue SVF cells

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Introduction

Adipose tissue is a highly active endocrine organ secreting a range of compounds, adipokines, with both local and distant actions. Adiponectin (Adq, Acrp30, AdipoQ, amp-1, GBP28) has been demonstrated to play an important role in the modulation of the liver glucose and lipid metabolism. On the contrary to leptin, the decreased plasma level of Adq is observed in patients with obesity and type 2 diabetes. Adq exerts anti-inflammatory and antiatherogenic properties. Modulation of the adiponectin level in plasma by nutrients (fatty acids, glucose) may support the normal blood/vessel wall homeostasis. The adipose stromal vascular fraction (SVF) cells play an essential role in the endocrine activity of adipose tissue. In this project the influence of FFA on expression of adiponectin during differentiation of the SVF cells was investigated.

Materials and Methods

Isolation and culture of human stromal adipose tissue progenitor cells (SVF) was done using modified Hauner method. After adaptation (5-7 days) in Adaptation Medium (SVF-AM, DMEM+insulin+hydrocorti sone+transferin) cells were stimulated to differentiate to adipocytes (SVF-Adipo), by 48h incubation with adipogenic factors: IBMX, insulin, dexamethasone and culturing in Adipo medium- (DMEM+insulin+hyd rocortisone+transferin+triiodothyronine) or to endothelial cells (SVF-Angio, by 24h incubation in Angio medium-EBM+2%FCS). Then cells (SVF-AM, SVF-Adipo, SVF-Angio) were for 24 h incubated with non-toxic (10-30 μM) concentrations of fatty acids (FFA): arachidonic acid-AA, eicosapentaenoic acid-EPA, oleic acid-OA and palmitic acid-PA). After incubation with FFA, the relative adiponectin gene expression was performed using real-time PCR which was followed by Western Blot analysis of adiponectin protein level. Changes in amount of adiponectin released to medium by differentiating SVF cells was measured using Quantikine Human Adiponectin Immunoassay (R&D Systems, Minneapolis, USA).

Results

In non-differentiated SVF-AM cells palmitic acid (PA) up-regulated adiponectin gene expression and markedly elevated the level of adiponectin protein (western blot) whereas eicosapentaenoic acid (EPA) down-regulated adiponectin gene expression and decreased the amount of adiponectin released by non-differentiating SVF-AM cells. Oleic acid (OA) upregulated adiponectin expression in SVF cells differentiating to endothelial cells (SVF-Angio) while PA decreased the amount of adiponectin released by SVF differentiating to adipocytes (SVF-Adipo).

Conclusion

Free fatty acids variously regulated the expression of adiponectin, depending on differentiation status of SVF cells. Saturated palmitic acid up-regulated adiponectin expression in non-differentiated SVF cells and down-regulated its level in cells differentiated to adipocytes. In turn, polyunsaturated omega-3 fatty acid/eicosapentaenoic acid inhibited adiponectin in non-differentiated SVF cells.

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N-3 polyunsaturated fatty acid effects on the cardio-metabolic risk in rats

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Introduction/objective(s)
This work aimed to evaluate the impact of dietary n-3 polyunsaturated fatty acid (PUFA), in particular according to their chain length, on cardio-metabolic risk prevention in two rat models of insulin resistance (IR) and hypertension. The first model was the fructose-fed rat in which metabolic syndrome is environmental, dietary-induced by the consumption of fructose-enriched food, and the second model was the spontaneous hypertensive rat (SHR), in which IR and hypertension developments involve the genetic background.

Materials and Methods
Fructose-fed rats and SHR were submitted to either α-linolenic acid (ALA) or long chain n-3 PUFA (ALA+EPA, eicosapentaenoic acid, +DHA, docosahexaenoic acid) intakes. During the experiment, glucose and insulin tolerance tests were performed and plasma triglycerides concentration was quantified. Arterial blood pressure (BP) was evaluated punctually by tail-cuff or chronically by implanted telemetry. After a 10-week period of diet, the FA profile of insulin-sensitive tissues (liver, skeletal muscle, heart, and adipose tissue) was analyzed by gas chromatography.

Results
Both models exhibited IR in the course of the experiment. This IR was linked to a specific FA pattern in insulin sensitive tissues. This pathological status was related to high cardiovascular risk as evaluated by the increase of BP and especially by the increase of pulse pressure in the fructose-fed rats. The n-3 PUFA-rich experimental diets prevented the changes of FA pattern in insulin sensitive tissues. The intake of the long chain n-3 PUFA prevented IR, impaired glucose tolerance, BP rise and hypertriglyceridemia. Conversely, ALA alone affected only hypertriglyceridemia.

Conclusions
The beneficial effects of n-3 PUFA on the whole cardio-metabolic risk factors in term of prevention were obtained only with the longer chain of the n-3 PUFA family. Part of this work has been already published (reference: Robbez Masson et al, *J Nutr* 2008 Oct; 138(10):1915-22).

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Enriching poultry meat with long chain n-3 fatty acids

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The benefits to cardiovascular health of long chain n-3 PUFA (LC n-3 PUFA) are well known but LC n-3 PUFA consumption is low. Poultry meat is widely consumed and its fatty acid composition can be manipulated by diet. LC n-3 PUFA consumption could, therefore, increase if poultry meat was enriched with LC n-3 PUFA. If the birds' diet is enriched with the relatively abundant and inexpensive C18:3 n-3, then the meat is also enriched with C18:3 n-3, with virtually no enrichment of LC n-3 PUFA in the edible tissues. Birds therefore need to be fed LC n-3 PUFA, from either fish oil or marine algae. There is no evidence that the source of LC n-3 PUFA affects the degree of LC n-3 PUFA enrichment in the edible tissues. Using 80 g fish oil/kg diet produced skinless white meat containing 420 mg LC n-3 PUFA in a 200 g serving. However, there are practical problems with formulating diets with this much oil. Fishy taints were also detected in cooked, reheated meat from birds fed >40 g fish oil/kg diet unless the vitamin E content of their diet was also increased to 200 iu/kg. The 'protoype' chicken that was developed was produced by feeding diets containing (/kg diet) 40 g fish oil and 100 iu vitamin E in the finishing phase. This produced skinless white meat with a LC n-3 PUFA content of 300 mg/200 g serving. If all conventional poultry meat were replaced with enriched meat in the EU, consumption of LC n-3 PUFA would be increased by approximately 80 mg/d and over 39 000 fewer lives may be lost each year in the EU.

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Effects of eicosapentaenoic acid on fatty acid oxidation and metabolic flexibility in human skeletal muscle cells

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Skeletal muscle is the main tissue in lipid and glucose oxidation in the body. Usually, glucose oxidation dominates in the fed state; however fat oxidation increases both during fasting and during sustained exercise. Metabolically healthy skeletal muscle is characterized by the ability to switch easily between glucose and fat oxidation, whereas inability to increase reliance upon fat oxidation seems to be related to the pathogenesis of insulin resistance in skeletal muscle (1, 2). Increased plasma free fatty acid (FFA) levels have been associated with insulin resistance (3). We therefore wanted to study the effect of pretreatment with fatty acid on fatty acid oxidation and metabolic switching in human skeletal muscle cells.

We have studied the effect of 24 h pretreatment with different fatty acids on acute (4 hours) 14C-oleic acid (OA) oxidation in cultured human skeletal myotubes with and without glucose present. We have defined metabolic flexibility as the ability to increase the acute OA oxidation while changing from the "fed" (low fatty acid, high glucose) to the "fasted" (high fatty acid, low glucose) state, adaptability as the ability to increase the acute OA oxidation with increasing OA concentration, and suppressibility as the ability of the cells to decrease the acute OA oxidation by glucose.

In human myotubes, 24 hours pretreatment with 100 μM eicosapentaenoic acid (EPA) significantly increased the flexibility, increased the adaptability and increased the suppressibility compared to 24 hours pretreatment with 100 μM OA, while palmitic acid (PA) had no effect. Linoleic acid (LA) significantly increased the suppressibility compared to OA, but had no effect on the other parameters. These results suggest a possible favourable effect of EPA on skeletal muscle metabolic switching.

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Individual fatty acids and inflammatory gene expression in endothelial cells: impact of cell stimulation state

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Background
Endothelial dysfunction is critical in atherosclerosis development and is often present in those with type 2 diabetes. A review of current literature has shown disparity in the reported *in vitro* effects of fatty acids using endothelial cell models. The present authors have highlighted the observation that reported anti-inflammatory effects of fatty acids are most frequently observed in studies using stimulated cells, whilst no such effects are reported in studies using unstimulated cells.

Objective
To perform the first systematic investigation of the effect of various fatty acids, representing a range of fatty acid subclasses, on the expression of a broad spectrum of genes associated with endothelial inflammation (ICAM-1, VCAM-1, E-selectin, MCP-1, Enos) under stimulated and unstimulated conditions to elucidate whether differences in methodological design, could explain reported differences in fatty acid effects.

Methods
Using human umbilical vein endothelial cells (HUVEC) the effects of DHA, EPA, linoleic, oleic and palmitic acids (100µm), +/- 10 ng/ml TNF, on gene expression were assessed by quantitative real time reverse transcriptase polymerase chain reaction (RT-PCR).

Results
The level of gene expression up-regulation, relative to control, was often attenuated in stimulated cells compared to unstimulated cells. Palmitic acid caused a near significant increase in the up-regulation of E-selectin gene expression in unstimulated compared to stimulated cells ($p=0.058$). MCP-1 gene expression was significantly down-regulated by DHA in unstimulated compared to stimulated cells. In contrast, linoleic acid caused significant increased up-regulation of MCP-1 gene expression in unstimulated compared to stimulated cells.

Conclusion
Fatty acid effects on inflammatory gene expression vary dependent on the fatty acid and cell stimulation state. Attenuation of effect in stimulated compared to unstimulated cells may be indicative of an adaptive response. These findings may explain disparity in current literature.

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Effects of dietary fat modifications on insulin sensitivity and metabolic markers of the metabolic syndrome. *Lipgene* – a randomised European dietary intervention study

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Objective
Dietary saturated fatty acids (SFA) may promote insulin resistance. *Lipgene* investigated the metabolic effects of substituting dietary SFA by replacement with monounsaturated fatty acids (MUFA) or as part of a low-fat (LF) diet in subjects with the metabolic syndrome.

Materials and Methods
417 free-living subjects with the metabolic syndrome were randomly assigned to one of four isoenergetic diets distinct in fat quantity and quality; high-SFA (HSFA); high-MUFA (HMUFA) and two low-fat, high complex carbohydrate (LFHCC) diets, supplemented with 1.24g/day of long chain omgea-3 PUFA (LC *n*-3 PUFA) or placebo for 12 weeks.

Results
Pre-treatment habitual dietary fat composition had a marked effect on markers of insulin sensitivity. The HSFA diet adversely affected insulin sensitivity, particularly in females (P=0.022), and increased Sicam concentrations (P=0.05) in subjects who habitually consumed a low-fat diet (<36% energy from fat). In contrast the HMUFA diet improved HOMA-IR and the insulin:c-peptide ratio in subjects who habitually ate a high-fat diet (P=0.024 and P=0.001, respectively). Furthermore omega-3 PUFA supplementation with a LFHCC omega-3 PUFA diet reduced plasma triacylglycerol (TAG) and non-esterified fatty acid (NEFA) concentrations (P<0.01), and the atherogenic index (P=0.03), an effect most evident in men.

Conclusions
The *Lipgene* intervention study showed that fat composition can modulate markers of the MetS which is dependent on habitual fat intake such that SFA had detrimental and MUFA beneficial effects on insulin sensitivity, particularly in women. The LFHCC omega-3 PUFA diet improved lipid related metabolic syndrome risk profiles particularly in men. Thus nutrition strategies can improve the metabolic phenotype of subjects with the metabolic syndrome within the context of an overweight/obese phenotype.

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Effect of dietary fat modification on skeletal muscle fatty acid handling in the metabolic syndrome; a stable isotope approach

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Introduction
In the metabolic syndrome, adipose tissue lipid buffering capacity may be disturbed, resulting in increased lipid overflow in the circulation and increased fat storage in 'non-adipose' tissue like skeletal muscle, which may be related to insulin resistance. Therefore, the aim of this study was to examine the effects of chronic dietary fat modification on the capacity of skeletal muscle to handle dietary and endogenous fatty acids (FA) in the metabolic syndrome.

Methods
In a sub-cohort of the *Lipgene* study, 39 men (58±2yr, BMI 30.3±0.7kg/m) with the metabolic syndrome were randomly assigned to receive one of four diets for 12 weeks: a high-fat saturated fat diet (HSFA), a high-fat mono-unsaturated fat diet (HMUFA), and 2 low-fat high-complex carbohydrate diets, either supplemented with placebo (LF) or 1.24g/d DHA/EPA (LF*n*-3). Effects of the diets on fasting and postprandial skeletal muscle FA handling were examined by measuring arterio-venous differences across skeletal muscle. Briefly, [H2]-palmitate was infused intravenously to label endogenous fat in the circulation and subjects received a high-fat mixed meal (providing 2.6 MJ, 61 E% fat, 35.5 E% SFA) containing [U-C]-palmitate to label chylomicron-TAG. Insulin sensitivity was measured with an insulin-modified intravenous glucose tolerance test.

Results
No differences were observed in insulin sensitivity nor in postprandial insulin and glucose concentrations between the diets. However, postprandial circulating TAG concentrations were significantly lower in the LFomega-3 group than the HSFA group and we observed lower concentrations of [U-C]-labelled TAG, representing total chylomicron-TAG. No differences were observed in skeletal muscle FA handling between the diets.

Conclusions
The LFomega-3 diet resulted in decreased postprandial TAG concentrations, which seemed to be due to a lower accumulation of dietary FA in the circulation. This is not accompanied by a higher clearance in skeletal muscle, suggesting that a higher uptake of chylomicron-TAG by adipose tissue or a lower intestinal TAG absorption may be responsible for this.

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Correlation of rumen microbial community structure with milk fatty acid composition

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Introduction: Biohydrogenation of fatty acids in the rumen converts health-promoting polyunsaturated fatty acids consumed by the grazing cow to less healthy saturated fatty acids, which eventually make up the majority of fatty acids in ruminant products, including meat and dairy products. The aim of this work was to try to understand how the microbial population of the rumen influences milk fatty acid composition.

Materials and Methods: Milk composition data from seven dairy trials carried out at the University of Reading and at MTT, Jokioinen, Finland, were compared with qPCR analysis of the main known biohydrogenating bacteria of the rumen using principal component analysis.

Results: Across-trial analysis indicated some correlations between concentrations of individual fatty acids in milk and the numbers of different bacterial species, but these were generally for minor components of milk, and no correlation was evident between the bacterial community and the main groups of fatty acids in milk, either individually or collectively. The basal diet was the main determinant of milk fatty acid composition across trials. Within individual trials, where the basal diet was similar, there was a stronger link between microbial community composition and fatty acid content of milk.

Conclusions: Strategies aimed at controlling milk fatty acid composition by manipulation of the microbial community in the rumen are likely to be less effective than altering the composition of the basal diet.

This work was supported by *Lipgene*, an EU Sixth Framework Programme Integrated Project (Project number: FOOD-CT-2003-505944).

Dietary supplementation of tetradecylthioacetic acid increases feed intake, but reduces body weight gain and adipose depot sizes in rats fed high-fat diets

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Aim: The pan-PPAR ligand and fatty acid analogue tetradecylthioacetic acid (TTA) may reduce plasma lipids and enhance hepatic lipid metabolism, as well as reduce adipose tissue sizes in rats fed high-fat diets. This study further explores the effects of TTA on weight gain, feed intake and adipose tissue functions in rats fed a high-fat diet up to 7 weeks.

Methods: The effects on feed intake and body weight during 7 weeks dietary supplement with TTA (~200 mg/kg bw) was studied in male Wistar rats fed a lard-based diet containing ~40% energy from fat. Adipose tissue mass, body composition and expression of relevant genes in fat depots and liver were measured at the end of the feeding.

Results: Despite enhanced feed intake, rats fed TTA gained less body weight than lard-fed rats and had markedly decreased subcutaneous, epididymal, perirenal and mesenteric adipose depots. The effects of TTA-feeding with reduced body weight gain and energy efficiency (weight gain/feed intake) started between day 10 and 13. Body content of fat, protein and water was reduced after feeding TTA, with a stronger decrease in fat relative to protein. Plasma lipids, including NEFA, were significantly reduced, whereas fatty acid ß-oxidation in liver and heart was enhanced in TTA-fed rats. *Ucp3* was expressed ectopically (>1900-fold) in livers, whereas *Ucp1* was increased ~30-fold in epididymal and ~90-fold in mesenteric fat after TTA-feeding.

Conclusion: Our data support the hypothesis that TTA-feeding may increase hepatic fatty acid ß-oxidation, thereby diminishing storage of fat in adipose tissues. The increased expression of hepatic *Ucp3* and of *Ucp1* in abdominal adipose tissues may together promote enhanced energy dissipation and reduced weight gain in rats.

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Lipgene – Diet, genomics, and the metabolic syndrome: an integrated nutrition, agro-food, social and economic analysis

Background to the Project

1.0 Introduction

Each year the number of obese Europeans is increasing, and this is having a serious impact on health. Obesity causes a number of metabolic disturbances that can adversely affect an individual's chance of suffering from other chronic diseases later in life. People who are overweight or obese are more likely to suffer from coronary heart disease, type 2 diabetes and high blood pressure. This clustering of risk factors is referred to as the metabolic syndrome. Within the UK alone, it is thought that as many as 25% of the population show clear signs of this syndrome, and there is little doubt that this is also the case in many European countries. Unless effective public health strategies are enforced, it is expected that the prevalence of the metabolic syndrome will continue to increase, bringing with it a dramatic rise in health and social welfare costs needed to treat the condition.

A note about obesity

Overweight and obesity are defined as 'abnormal or excessive fat accumulation that presents a risk to health' . The Body Mass Index (BMI) is one of the most common tools used to define obesity.

$$BMI = \frac{\text{weight (kg)}}{\text{height (m)}^2}$$

A person with a BMI of over 25kg/m^2 is said to be overweight, and those with a BMI greater than 30kg/m^2 obese.

Whilst the prevalence of obesity within Europe has been on an upward trend for some time, there is evidence to suggest that this increase has become more marked in recent years[3]. Within the 15 countries that made up the EU in 2002, an estimated 18% of men and 21% of women were obese. This varied within countries, with Austria topping the list with an alarming 30% of men and 26% of women being obese. Conversely, the rates in the Scandinavian countries were relatively low (approximately 15% of men and 16% women). Projecting current obesity trends to 2025, it is estimated that a staggering 30% of men and 34% of women will be obese unless something is done to halt this rise[4]. Within the UK, the government predicts that if current trends continue, by the year 2050 over half of the population will be obese[5]; there is little doubt that similar trends will not be seen across all EU Member States.

Not only does obesity seriously impact upon the mental wellbeing and quality of life of those affected[6], it also predisposes them to a number of chronic diseases, which together constitute the principle causes of death in the developed world[7]. These include: cardiovascular disease (CVD) (which includes coronary heart disease (CHD) and stroke); cancer[8]; and type 2 diabetes[9].

Obesity cost the European economy an estimated €32.8 billion in 2002. This figure includes the costs associated with treating the complications of obesity and lost productivity from days taken off sick. Unless something is done to stop the predicted increase in the prevalence of obesity in Europe, the consequences for both health and the economy are likely to be severe. Obesity and its related complications may end up costing the EU up to 6% of its health care budget by the year 2025[4].

2.0 The Metabolic Syndrome

The metabolic syndrome (or syndrome X) is a term used to describe a cluster of metabolic disorders which increases an individual's risk of suffering from CVD and type 2 diabetes[10]. These disorders commonly include insulin resistance, hyperglycaemia, central obesity and hypertension.

Currently, no accepted definition of the metabolic syndrome exists, although a number of different diagnostic criteria have been proposed by different organisations[11, 12, 13].

Despite this lack of a standard definition, central to all diagnostic criteria is the fact that sufferers experience some degree of impaired glucose tolerance and insulin resistance. Further symptoms of the syndrome may also include:

- Central/abdominal obesity
- Raised blood pressure/hypertension
- Dyslipidaemia (abnormal blood lipids)
- Gout
- Abnormalities in blood clotting
- Low cardio-respiratory fitness
- Presence of fatty liver disease or polycystic ovary syndrome

A note about insulin – Insulin is a hormone produced by the pancreas in response to food intake. Insulin circulates in the blood and regulates the movement of glucose into cells. Once inside the cell, glucose is used as an energy source, or stored as glycogen until needed.

Insulin resistance is a condition whereby the body's cells are less sensitive to the action of insulin. It occurs when insulin is secreted as normal by the pancreas, but it does not carry out its usual function as outlined above. As a result, the amount of glucose in the blood becomes too high (hyperglycaemia). To try and overcome this problem and maintain normal blood glucose levels, the pancreas secretes additional insulin but, for some people, the body's cells still do not respond. This situation leads to insulin resistance, and type 2 diabetes often consequently develops from prolonged elevated blood glucose levels.

The underlying cause of the metabolic syndrome is unknown, but insulin resistance and obesity are thought to be key. Possible theories have included the idea that insulin resistance may be the primary event leading to its onset. However, other theories suggest that it is the onset of obesity that is responsible for disturbances in carbohydrate and lipid utilisation in tissues, and insulin resistance is second to this[10].

Whatever the initial cause, the rising obesity levels seen in EU countries consequently result in a population at an increased risk of developing type 2 diabetes – indeed, approximately 80% of people diagnosed with type 2 diabetes are overweight[14]. Due to a lack of a universally accepted definition for the metabolic syndrome, estimating its prevalence is often difficult. However, in the US, data from the National Health and Nutrition Examination Survey show that the prevalence of the metabolic syndrome increased from 23.1% in the period 1988-1994, to 26.7% in 1999-2000[15]. It is thought these figures may be an underestimation of the actual prevalence as, due to the complex process involved in the development of the disease, many cases are believed to go undiagnosed[16].

Within the UK, it is thought that as many as 25% of the adult population show clear signs of the syndrome[1] and, due to the fact that the burden of obesity may be the driving force behind the metabolic syndrome, it is likely that the prevalence within the population will increase in parallel with the rising incidence of obesity. Within a population, there is a marked difference in prevalence between different subgroups. The risk of developing the metabolic syndrome increases with age, with the metabolic syndrome chiefly being thought of as a disease of middle and old age[17]. In more recent years however, cases of children showing signs of type 2 diabetes have been emerging[18]. Another subgroup of the population at an increased risk of the syndrome is certain ethnic subgroups. This is exemplified in a study which found the prevalence of the syndrome to be higher in South Asians when compared to Europeans[19].

3.0 Dietary Fat

Dietary fat is essential for overall health and wellbeing.

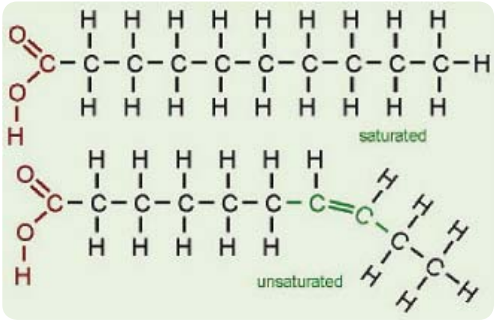
Why we need fat

- It acts as an energy source for the body
- It enhances the flavour and palatability of food
- It is the source of essential fatty acids
- It acts as a carrier for fat soluble vitamins

However, dietary fat, the most energy dense nutrient (providing 9kcal/g) can make a substantial contribution to energy intakes and may influence the development of various chronic diseases, including obesity and the metabolic syndrome. It is for this reason that getting the correct amount and type of fat in the diet is critical if we are to reduce the burden of these chronic diseases.

The fatty acid chains which make up dietary fat comprise a chain of carbon atoms, with a methyl group at one end and a chain of carbon atoms at the other. The nature of the fatty acids, and therefore the fat, depend upon whether these carbon atoms are saturated with hydrogen, or not (unsaturated).

All fats and oils regardless of their source comprise a mixture of different types of fatty acids, but the ratio of the different types varies.



Unsaturates can be further subdivided into two types: monounsaturates (MUFA) and polyunsaturates (PUFA). MUFAs are characterised due to the presence of one double bond in their carbon backbone, whereas PUFAs may have two or more double bonds.

PUFAs can be further categorised, with the two main groups being the omega-3 (or *n*-3) and omega-6 (or *n*-6) fatty acids. Two particular fatty acids, alpha-linolenic acid (omega-3) and linoleic acid (omega-6) are termed 'essential' fatty acids, as they cannot be synthesised by the body, and therefore must be provided by the diet.

3.1 Health Effects of Dietary Fat

The amount and type of fat in the diet influences cardiovascular disease risk, as saturates and unsaturates have different effects on the balance of cholesterol carrying proteins in the blood[20].

Diets high in saturates ↑ cardiovascular disease risk, as they ↑ the amount of total and low density lipoprotein cholesterol in the blood.

Diets high in unsaturates ↓ cardiovascular disease risk, as they ↓ the amount of total and low density lipoprotein cholesterol in the blood.

Whilst it has been recognised for some time that an adequate intake of omega-6 PUFAs is important for reducing the risk of CVD, there is now an increasing amount of evidence suggesting that long-chain omega-3 PUFAs are also important for health and disease prevention.

Long-chain omega-3 PUFAs are synthesised by the conversion of alpha-linolenic acid (ALNA) to eicosapentanoic acid (EPA) and then to docosahexanoic acid (DHA) via a process of elongation (addition of carbon units) and desaturation (addition of a double bond to replace a single bond between neighbouring carbon atoms) of the long-chain omega-3 PUFA. These fatty acids play differing but essential roles within the body: DHA is a major constituent of membrane phospholipids, enhancing their fluidity, and metabolites of EPA form the basis for many regulatory signals. The main dietary source of these long-chain omega-3 PUFAs is oily fish. However fish, like humans, cannot synthesise these important fatty acids. Instead they accumulate them from the food they eat; the organisms responsible for the presence of EPA and DHA in the food chain are marine algae at the bottom of the marine food chain. Current evidence suggests that the benefits of consuming long-chain omega-3 polyunsaturates include reductions in blood pressure and blood triglyceride concentrations[24], as well as favourable effects on blood clotting and anti-arrythmic effects[21].

3.2 Fats in the Human Food Chain

In the past several decades we have witnessed very considerable changes in the composition of dietary fat to a more nutritional optimal balance of fatty acids. Initially this involved a reduction in saturates with a partial replacement by n-6 polyunsaturates. The initial view that monounsaturates were "neutral" with respect to blood lipids was challenged in the late 1980s and 1990s and it was during that time that the importance of long chain n-3 PUFA became evident. The global rise in obesity led to the development of lower-fat varieties of foods and in more recent times, there has been a rapid removal of industrially hydrogenated trans fat from the human food chain.

Changing dietary patterns have also had an impact on the fatty acid profile of the diet – as cooking oils have replaced harder fats such butter in the diet, intakes of saturates have fallen and intakes of mono- and omega-6 polyunsaturates have increased.

However, intakes of omega-3 PUFAs have lost some ground as fish oil has been replaced in a number of applications by vegetable oils, and this has been further compounded by the changing dietary pattern in relation to meats. Meat from ruminant animals can make a substantial contribution to intake of ALNA, but as a result of the falling consumption of lamb and beef over the past two decades and the changes in animal feeding practices, away from grass, meat and dairy products now make a smaller contribution to the amounts of ALNA in the diet.

Despite recent emphasis on the heart health benefits of the long-chain omega-3 PUFAs, intakes remain far short of the recent recommendation for the UK of 450 mg/d[22]. However, this varies between countries in Europe – whilst intakes of the long-chain omega-3 polyunsaturates are low across most of mid-Europe owing to a substantial proportion of the population preferring not to consume oily fish, intakes in Southern and Northern Europe are higher, reaching or exceeding the level of 450 mg/d established in the UK.

These changes in the level and composition of the fats in the human food chain are rapidly reaching the point of diminishing returns. The easier changes have been made from lower fat dairy products to low-fat, low saturated fatty acid spreads. The next generation of change will require us to manipulate the primary source of dietary fats – those arising from farmed animals and those from cultivated crops. That challenge is one faced by those involved in animal husbandry and nutrition and by the world of plant biotechnology.

4.0 The Association between Dietary Fat and the Metabolic Syndrome

Insulin resistance is often described as the underlying factor in the development of the metabolic syndrome. Resistance to insulin can be brought about by different environmental factors, one of which is diet. Consumption of energy dense/high fat diets has been shown to be positively associated with overweight which, in turn, causes insulin sensitivity to deteriorate[23].

Insulin sensitivity may also be affected by the type of dietary fatty acid, with epidemiological evidence and intervention studies clearly showing that saturates significantly worsen insulin-resistance[24] and diets rich in monounsaturates and polyunsaturates may improve it[25,26,27].

Lipgene scientists set out to add to the evidence base in this area, by evaluating what happens to people at risk of the metabolic syndrome if they change the balance of fatty acids in their diet. Specifically, they aimed to provide answers to questions such as: 'how much of an improvement in metabolic abnormalities is possible with dietary modification alone?' and 'are some people more sensitive to certain types of fat than others?'

As is apparent from the aforementioned studies, dietary fat appears to play a causative role in the development of the metabolic syndrome. In general, the dietary advice given in studies such as those above reflect those suggested for the general population: reduce energy from total fat and saturates, eat plenty of starchy carbohydrates, and increase consumption of fruit and vegetables[28]. Due to the positive effects seem with consumption of a Mediterranean diet (high in nuts, fruit and vegetables, wholegrains and fish) on numerous metabolic risk factors associated with the metabolic syndrome, this type of dietary pattern is often also suggested for those affected by the condition[29].

5.0 Food of the Future – Social and Economic Aspects

The next several decades will see some major challenges to the global food supply as the worlds' population grows by 50% and as global warming reduces agricultural output in many regions of the world. Without doubt, this will lead to a new era of innovation in primary agricultural production and in new food product development. However, increasingly, options for change in the human food chain will be subject to screening for their economic impact and their social acceptability. Innovations will not be judged solely by their ability to optimise human physiology. They will also be judged by their population impact and thus a modest change reaching the majority of a population will be seen differently to a very significant change which will impact on only a few. Thus the population economic benefit will be linked to the economic costs of development and the latter may be subject to subsidies to favour innovation with a wide population impact. Innovation must also be socially acceptable and increasingly we will see consumer research influencing all stages of food innovation from original concepts right through the marketing.

6.0 Genetics and the Metabolic Syndrome

Whilst dietary factors play a large part in the development of the metabolic syndrome, the inherited nature of this disease[30], coupled with the marked differences in prevalence of the disease between different ethnic goups[31], and the concordance rates seen between monozygotic twins[32], indicate that genes are likely to also play a large part in its etiology.

Until recently, dietary recommendations for disease prevention have taken a one-size-fits all approach. However, in recent years there has been an increasing focus on the possibility that genetics may play a role in an individual's response to diet. Research seems to point to the fact that an individual's genetic make-up may indeed influence many different aspects of nutrition, including appetite, food choice, and nutrient absorption and subsequent metabolism[33, 34].

Nutrigenomics is the name which has been given to this relatively new area of research, and the *Lipgene* project is one of the first to try and understand how an individual's genetics may influence the effect of diet on the development of the metabolic syndrome.

Given the complex nature of the metabolic syndrome, identifying potential candidate genes that may be involved in its aetiology is complex. The candidate-gene approach, which identifies genes according to biological function and linkage studies, is often used[35].

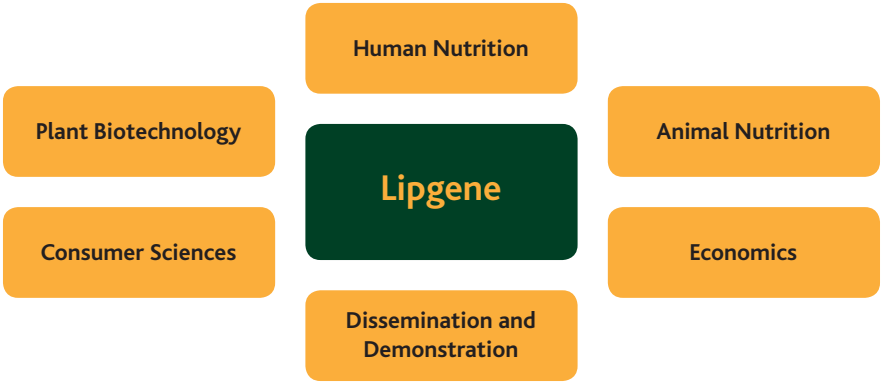
Epidemiological studies have thus far shown varying effects of genetics on dietary intake. In terms of the metabolic syndrome, our attention turns to the possible role of the PPARγ gene, which has been identified as a possible candidate gene for conferring susceptibility to the metabolic syndrome. This is due to the fact that this gene encodes a transcription factor which regulates adipogenesis, lipid and glucose metabolism[36]. It has been shown that a common allele of this gene is associated with a modest but significantly increased risk of type 2 diabetes[37]. Different alleles of this gene have also found to be associated with differing effects on metabolic risk factors following consumption of dietary fat[38].

7.0 *Lipgene*

Although the exact cause of the metabolic syndrome is unknown, new scientific technologies are allowing researchers to investigate links between our diet and genes, in a bid to gain a greater understanding about the onset and development of this chronic disease. Further, consumer science research into this area will allow for determination of particular lifestyle habits that may be associated with disease onset.

Effective strategies are also needed to decrease the dependence of those suffering from the syndrome on medical management. Agro-food technology and genetically modified (GM) foods are two such options. However, past opposition by consumers to the use of these technologies may impact upon their potential to help prevent and manage this disease.

Lipgene is an Integrated Project of the EU Sixth Framework Programme for Research and Technological Development. The project is being conducted by 25-research centres in fourteen countries across Europe. The *Lipgene* project, entitled 'Diet, genomics, and the metabolic syndrome: an integrated nutrition, agro-food, social and economic analysis' began in 2004, and will finish in January 2009. It set out to examine and understand the metabolic syndrome, and to provide a multidisciplinary approach to its effective management and prevention. The project adopts a truly integrated approach to examine these points, with six workpackages set up to investigate different areas of the project.



Aim: to gain an understanding of the factors involved in the development of the metabolic syndrome, and to provide an approach to its effective management and prevention. More specifically:

- To understand the manner in which differences in the composition of dietary fat interacts with natural human genetic variation to influence the development of the metabolic syndrome
- To create alternative plant sources of long chain omega-3 polyunsaturates, by taking genes from marine algae to produce a seed-oil containing the vital long-chain omega-3 PUFA usually found in fish oil
- To establish the principles of animal nutrition, which change the composition of milk fat from dairy cows to one with less saturates, less *trans* fats and more monounsaturates. In addition, animal nutrition research will increase the supply of long-chain omega-3 PUFA enriched poultry meats to provide additional sources of these fats
- To examine the economic barriers to introducing new agro-food technologies and the economic costs of the management of the metabolic syndrome through diet versus pharmaceutics
- To ascertain the concerns and views of the consumer as to the risks arising from the metabolic syndrome and the attitudes regarding the risk benefits of introducing new agro-food technologies to combat the metabolic syndrome
- To increase consumer awareness about the metabolic syndrome and associated health risks
- To complete a wide-ranging and high-level dissemination programme to: create awareness of the need to integrate diet and genetics in addressing the metabolic syndrome; and create awareness of the potential of new agro-food technologies to help combat the metabolic syndrome
- To provide a greater availability of food products that can enhance human health

In the following sections, each of these different areas of research is focused upon in more detail.

7.1 Human Nutrition Studies

The human nutrition intervention arm of *Lipgene* is composed of three main strands, all of which are discussed below.

A Prospective Cohort

The first part of the human nutrition studies involves the use of a pre-existing cohort of subjects to track the development of the metabolic syndrome. As the clinical end-points of this study are relevant to the interaction between genes and the metabolic syndrome, the cohort has been used in *Lipgene* to retrospectively study the development of the metabolic syndrome and diet-gene interactions.

The SUVIMAX study

- Short for: SUppléments en VItamines et Minéraux AntioXydants (Antioxidant Vitamin and Mineral Supplements)
- Began in 1994
- 13,000 participants, men and women aged 35-60 years living all over France
- Aim: to evaluate the effect of antioxidants on coronary heart disease and cancer incidence.

In 2001-2002 *Lipgene* identified 877 cases of the metabolic syndrome within this cohort, and 877 matched controls were also found. Genetic characteristics, baseline dietary analysis and baseline plasma fatty acid levels were amongst some of the markers compared between the groups, in a bid to identify potential dietary and genetic variables associated with the metabolic syndrome.

Generally, cases had lower levels of educational attainment that controls, and were more likely to be smokers and less physically active. They also had a higher mean BMI at baseline, and had higher levels of fasting glucose and insulin (risk factors for the metabolic syndrome) and lower fasting HDL-cholesterol. Comparison of dietary intake showed that cases had a lower total daily energy intake at baseline than controls, to which the contribution of alcohol and protein to total daily energy intake were higher and carbohydrate lower in cases than controls. The contribution to energy intake of lipids and saturates between the two groups was similar, although cases were found to have slightly higher intakes omega-6 PUFAs than controls.

The cases and controls were also genotyped, and genetic analysis undertaken to try and identify any genes, and pertinent single nucleotide polymorphisms (SNPs) within each gene, which may be associated with the development of the metabolic syndrome. Based on a list of 182 candidate genes and 806 SNPs initially being identified, 7 SNPs in 6 genes were found to be significantly different between subjects with the metabolic syndrome and matched controls. 3 of them, related to lipid metabolism or inflammation, seem particularly implicated. Due to the complexity of this disease, more powerful data analyses are on-going to better define effects of dietary or genetic factors."

A Dietary Intervention

The *Lipgene* human dietary intervention study was conducted to determine the effect of reducing intake of saturated fat (either in terms of reducing quantity or exchanging for MUFA) on multiple metabolic and molecular risk factors of the metabolic syndrome. Key metabolic markers determined included insulin sensitivity, lipid metabolism in the postprandial state, markers of inflammation, coagulation and vascular function, and cellular energy status. The second aim was to identify if common genetic polymorphisms involved in the metabolic syndrome determined whether an individual responded to dietary therapy.

Overall, 417 volunteers from eight cities across Europe, completed a 12-week intervention were they were randomised to one of four diets: A: high-fat, saturate-rich (HSFA); B: high-fat, monounsaturate-rich (HMUFA); C: low-fat, high-complex carbohydrate (LFHCC); and D: LFHCC with with long-chain n-3 PUFA. Dietary analysis showed that subjects adhered well to the interventions. Following the 12-week dietary intervention period, it was shown that subjects following both LFHCC diets lost a small amount of weight (<1 kg), despite the isoenergetic intervention. In the full cohort lowering SFA did not affect insulin sensitivity. However, when the effect of background diet on responsiveness to dietary intervention was examined there was a clear adverse effect of SFA on insulin sensitivity in individuals who habitually consume less than 36% of energy from fat. Also the LFHCC n-3 PUFA diet significantly improved several indices on lipid metabolism. On-going genetic bioinformatic analysis highlights important inflammatory genes which interact with dietary fat to determine insulin sensitivity – which indicates that genes can alter the effect of dietary fat modification.

Mechanistic Studies

The final part of the human nutrition studies aimed to investigate the role that dietary fatty acids play in the basic biology of the metabolic syndrome using animal and cell models. State of the art biology technology has been used to investigate the role of numerous different cellular processes in the development of the metabolic syndrome. These include:

1) the influence of fatty acid composition on adipogenesis – a key process involved in the development of insulin resistance and metabolic syndrome.

2) the role of fatty acids on human skeletal muscle cell fatty acid uptake– skeletal muscle is the primary organ involved in glucose disposal.

3) Atherogenesis and hypertension are both associated with insulin resistance, and endothelial function is a key regulator of these processes.

Publications arising from these studies have been listed at the end of the report. A number of students and post doctoral researchers presented posters on these studies at the conference in Dublin (5th Dec 08). The abstracts are listed at the beginning of this booklet.

7.2 Plant Biotechnology

The ultimate goal of the plant biotechnology group of *Lipgene* is to establish a sustainable source of long-chain omega-3 PUFAs for human consumption. Whilst oily fish provides a valuable source of these compounds in the diet, consumption of fish is low in many parts of the EU[39], and there are concerns over long-term sustainability. Therefore, scientists in this arm of *Lipgene* aimed to investigate the potential of genetic engineering (see appendix 1 for more information) to create transgenic plants which contain a substantial proportion of long-chain omega-3 PUFAs. Producing long-chain omega-3 PUFAs in seed oil has many advantages over fish oil, including: a lower odour; less environmental contamination; and the fact that plants are a sustainable and cheap source of fatty acids.

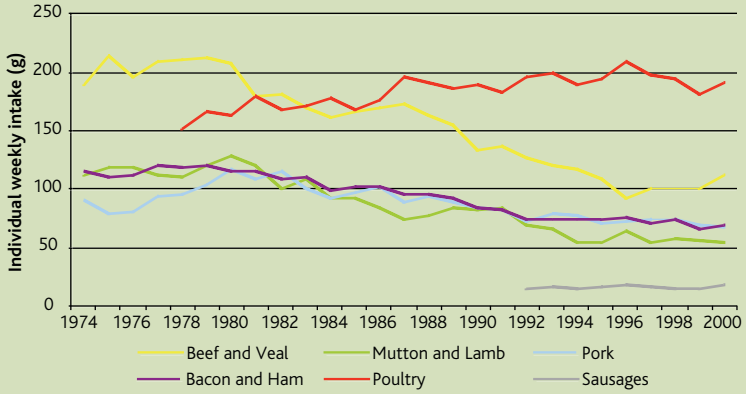
Using genes that regulate the production of EPA and DHA in marine algae, it was demonstrated that the omega-6 PUFA arachidonic acid (AA) and EPA could be produced in linseed. However, at only 5%, the concentration of these long-chain PUFA in the seed oil was too low for commercial use. Further, the ratio of omega-6 and omega-3 in these seeds needed to be optimised (a 5:1 ratio of omega-6:omega-3 is thought most beneficial for human health). Scientists working in this area have identified genes within rapeseed that are responsible for this 'bottle-neck' in the synthesis of long-chain PUFAs, and new genes associated with the biosynthesis of long-chain omega-3 PUFAs have also been identified. This has enabled the production of a strain of rapeseed oil with a fatty acid composition optimised towards omega-3 fatty acids, and also allowed an increased yield of omega-3. As well as producing these modified oils for human consumption, the possibility of using these oils in animal feed as part of the animal nutrition work was also discussed, with a view to enhancing the fatty acid profile of meat products by using a sustainable source of omega-3 rich oils (see below).

7.3 Animal Nutrition

There is a general consensus amongst scientists and health care professionals that diets high in saturates increase an individuals risk of suffering from the metabolic syndrome[24]. Animal derived fats, including milk and dairy products, and meat and meat products, are often found to be the greatest contributor of saturates in the diet[40].

However, whilst these animal derived foods are a major contributor to the population's intake of saturates, they also make a significant contribution to intakes of monosaturates and other key nutrients. In addition there is good epidemiological evidence that milk has some cardioprotective effects. Therefore, reducing their intake is not always desirable. The initial aim of the animal nutrition strand of *Lipgene* is to improve the dietary fatty acid profile of European consumers by modifying the fat content of two different foods: milk and poultry meat. For milk, the aim is to increase the content of monounsaturates, whilst simultaneously reducing the content of saturates, and at the same time avoiding or minimising an increase in *trans* fatty acids. Poultry meat was chosen as another potential vehicle to improve the fatty acid profile of EU consumers for a number of reasons: the meat has gained consumer and health care professional approval in recent years due to the fact it is lean, yet contains significant amounts of mono- and polyunsaturates. This is reflected by the fact that, whilst the consumption of ruminant meat has fallen significantly over the last decade, consumption of poultry meat has substantially increased.

The changes in meat consumption in the UK over the past 40 years



Source: DEFRA 2007 [41]

Past attempts to enrich poultry with EPA and DHA have often relied upon fish oils. However, with this comes a concern about reduced shelf-life of the meat, problems with sensory properties of the poultry, and a worry over the long-term sustainability of fish oils for use for this purpose. To overcome these problems, *Lipgene* scientists are currently trying to enrich poultry meat using unique sources of EPA and DHA, which will need to have a satisfactory oxidative stability and an acceptable taste.

The animal nutrition work has progressed well over the five years. Alteration of the fatty acid content of milk has now been achieved. However the reduction in saturates and increase in monounsaturates has also come with a small but possibly undesirable increase in the *trans* fatty acid content. This problem will continue to be investigated further.

With regards enrichment of poultry meat, whilst the initial plan was to use enriched rapeseed oil produced in the plant biotechnology workpackage as feed for the chickens, the amount of oil produced from this workpackage was small, therefore an alternative source of EPA and DHA was sought. Marine algae, the source of long-chain omega-3 polyunsaturates in the aquatic food chain, was the chosen alternative. Enrichment of poultry meat using this source of EPA and DHA has been achieved, and results show that this meat has the potential to meet recommendations for population omega-3 and omega-6 intakes if consumed at current levels. There is also the potential to further enrich this poultry meat beyond the target initially set, and this continues to be investigated.

7.4 Economics

As part of this workpackage, consortium members aimed to assess the direct and indirect costs of the metabolic syndrome, and to identify potential savings that could be made as a result of interventions identified by *Lipgene*.

Direct costs include staff time treating patients with the metabolic syndrome and the cost of drugs for treatment, and indirect costs associated with the metabolic syndrome include time off work due to illness, and premature death. There are significant health care costs associated with obesity, with estimations suggesting that, in 2002, the cost of obesity and its associated conditions in the EU (then 15 member states) was around €32.8 billion[4]. By modifying the type and quantities of fats found in foods, it is hoped that EU consumers can modify their fatty acid intake, and thereby reduce the risk of getting diseases such as the metabolic syndrome.

However, these modified (often called 'functional' foods) are expensive to produce and the products are therefore sold at a premium. Reasons for this high cost include: the higher cost of the ingredients needed to produce the food (i.e. feeding fish oil to cows); the additional cost imposed on farmers; and the need for dedicated supply chains for these products so they do not become contaminated with other products. Estimations suggest that these functional foods could cost between 35-85% more than their standard counterparts. As consumers may not be willing, or maybe unable, to pay these increased prices, subsidising these foods may be necessary. The cost of this would therefore need to be offset against the potential healthcare savings that would be made as a result of consuming these products. Estimates from this strand of *Lipgene* indicate that subsidising the cost of purchase of these healthier foods would be economically favourable when compared to the cost incurred by the metabolic syndrome.[4]

7.5 Consumer Understanding and Awareness

The consumer science work of *Lipgene* has two main points of focus: 1) to construct a psychometric model of the metabolic syndrome, demographic, lifestyle and psychological factors;[2]) to conduct a quantitative survey of consumer attitudes to interventions to prevent and treat the metabolic syndrome. Focus groups and interviews involving EU consumers and stakeholders were also held to explore these aims.

Evidence is accumulating to suggest that the metabolic syndrome may be triggered and/or exacerbated by psycho-social factors. How these factors interacted in the aetiology of the metabolic syndrome was relatively unclear, therefore *Lipgene* aimed to identify a model to determine causal links between the metabolic syndrome and these psychological risk factors. A representative survey of late middle-aged people was conducted in Portugal (N+500) and Great Britain (N=1000). The survey included a range of validated psychometric scales to measure resilience, stress, depression and mood. Enquiry was made of physical activity, dietary habits and life events. Anthropometric measures were also taken. Findings indicate that people who are older, inactive, have experienced negative life events, and who are less resilient, are more likely to have the metabolic syndrome.

Consumer perceptions of the metabolic syndrome and associated health issues, as well as attitudes towards potential interventions including functional foods, GM technology and personalised nutrition, were evaluated in a 6-country survey[42]. of nearly 6000 participants in France, Great Britain, Germany, Italy, Poland and Portugal. There was a good awareness of diet-related diseases, although only 31% knew of the term metabolic syndrome. When asked to select from a list of 'functional foods', those found to be commonly consumed included fruits and vegetables, with a quarter of the sample claiming to drink probiotic yoghurt drinks. Whilst past research from this area have shown consumers often have a negative attitudes towards GM foods, this survey found that one-third of participants would accept GM foods if they provided health benefits. Tests investigating the acceptance of genetic testing found that two-thirds of participants were willing to have a genetic test to identify their risk of type 2 diabetes/heart disease[43, 44].

7.6 Dissemination

An essential part of the *Lipgene* work is disseminating the findings of the project to the general public. The British Nutrition Foundation is responsible for this part of the *Lipgene* project, and information from the project has been presented in many different ways. These include the production of a bi-annual newsletter; the continuous updating of information on the *Lipgene* website, and the production of numerous articles in varying health professional and parliamentary magazines. Over the five year project, the dissemination strand has also hosted two conferences each year, where delegates are invited to hear from *Lipgene* scientists about the latest findings in their area of work. The most recent conferences included a conference in London (November 2007) to communicate the agro-food technology aspects of the project (plant biotechnology and animal nutrition), prior to which a press conference was held, creating worldwide coverage; and a 2-hour satellite meeting in Nottingham, in June 2008.

7.7 Demonstration

The demonstration project combines knowledge obtained from the activities in nutrition, agro-food technology and consumer understanding to create lipid-modified edible food prototypes with health benefits to the metabolic syndrome, which also have acceptable sensory characteristics, and to develop communication routes by which the benefits of these modifications can encourage consumers to exchange usual foods for these enriched ones.

A total of five food products were made with a modified fatty acid profile. These are: EPA/DHA enriched poultry meat and milk, cheese with reduced saturates and enhanced monounsaturates, two spreads (one 25% fat spread with 500mg EPA and DHA per 20g serving, and one 25% fat spread with 2g ALNA per 20g serving) and a salad oil with 2g ALNA and 500g EPA and DHA per 14g serving.

Lipgene scientists also investigated the impact of replacing regular foods with the enriched food prototypes on population intakes of omega-3 PUFAs. This was estimated using dietary data from the UK, the Netherlands and France. As may be expected, it was shown that the availability of food enriched with ALNA, EPA and DHA could help people achieve optimal omega-3 PUFA intakes; however the type of food enriched must be common, everyday foods.

With regards to the sensory characteristics of these products, results from sensory testing showed the spreads and oils enriched with omega-3 fatty acids had a shelf-life of 12 weeks, with sensory differences unlikely to be perceived by consumers. Changing the fat composition of the cheese had a significant effect on the sensory properties however the perceived difference is relatively small. Further, enrichment of poultry with EPA and DHA does not compromise the sensory characteristics, even when the meat is reheated.

An online consumer study was conducted to evaluate promising communication routes on the metabolic syndrome and the enriched food prototypes. Results showed that the metabolic syndrome is not something consumers reject but rather a preferred communication message. The products also became more acceptable to consumers when the communication was negatively framed e.g. "avoiding the metabolic syndrome" rather than "reaching a positive health situation".

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Appendix 1: Genetically Modified Foods

What are GM foods?

GM foods are those that have been genetically modified. Genetic modification is a process by which the genetic constitution—such as number and arrangement of genes—of an organism is altered. Genes are sections of DNA which carry instructions for expressing proteins, which in turn give a particular organism certain characteristics.

Why are foods genetically modified?

Modifying foods in this way allows food producers to select for desirable qualities in foods, and to avoid characteristics that are not desirable. For example, genetic engineering may be used to produce crops which can grow in unfavourable conditions (eg. drought).

How are foods genetically modified?

Producing organisms with desirable characteristics has traditionally taken place by a method called 'selective breeding'. Selective breeding occurs when animals or plants with the most desirable characteristics for use as food and feed are chosen for breeding the next generation. Those characteristics are found in naturally occurring variations or in artificially obtained mutants of those animals/plants. However, this is a fairly slow process, and because of then nature of gene distribution during reproduction, the desired results may take a few generations to be achieved. Therefore, genetic engineering is becoming a preferred favourite. This involves obtaining the DNA sequence of a desirable gene from an organism, amplification or chemical synthesis of this gene and insertion of this gene into the genome of another organism. This enables the target organism to express protein responsible for a certain desired characteristics in the next generation.

How is the *Lipgene* Project using GM technology?

Scientists working on *Lipgene* are working on modifying the fatty acid profile of different foods. In one project, scientists are trying to increase the amount of long-chain n-3 polyunsaturated fatty acids (PUFA) in vegetable (rapeseed) oil. Using genetic engineering technology, the genes involved in the synthesis of omega-3 polyunsaturates in marine algae will be used to develop a rapeseed oil with omega-3 polyunsaturates that traditionally only occur in marine foods.

What will this rapeseed be used for?

The ultimate goal is to increase the entry of omega-3 fatty acids into the human food chain. Whether this is best be done indirectly by enriching fish, pig or poultry with omega-3 fatty acids by feeding them an omega-3 LC-PUFA plant oil, or directly by adding this plant oil to products such as yogurt, is subject for future research.

Will this affect the taste of the foods in any way?

Taste is a very important factor determining people's food choices. It is anticipated that the taste of the foods enriched in plant produced omega3 LC-PUFA, and all GM foods in general, will not be affected.

What are the main benefits of GM technology?

GM foods offer many benefits. One of the most promising uses of GM foods are feeding the growing world population, by allowing crops to be produced which are disease, pest and drought resistant, and can therefore grow in unfavourable conditions. Further, in countries where malnutrition is prevalent, genetically modifying foods to enhance their nutrient content may help to alleviate this problem.

Are there any problems with GM technology?

Although GM foods have many promising benefits, many people are opposed to this technology. A variety of concerns exist about GM foods, including the unknown effects their consumption may have on human health, unintentional harm to wildlife, and unforeseen, adverse effects to the ecosystem.

So are these foods safe to eat?

Studies into the effects of GM foods on human health have shown that they are as safe as non-modified foods, and consumption poses no harm to human health. All GM foods which are sold worldwide are safety checked and must be approved as safe for consumption.

How can foods which have been genetically modified be identified by consumers?

Consumers can identify GM food products by looking at the label. In 2004 new laws came into force stating that foods which contain genetically modified organisms (GMOs) or ingredients produced from GMOs must be indicated on the labels. Foods produced with GM technology (e.g. cheese produced with GM enzymes) and products such as meat, milk and eggs from animals fed on GM animal feed will not have to be labelled.

Appendix 2: Obesity in Europe – A fat price to pay?

What is obesity?

Obesity is a condition in which abnormal or excessive fat accumulation in adipose tissue impairs health. It is defined in adults as having a body mass index (BMI) above 30. Obesity is one of the most visible but, until recently, most neglected public health problems. Body weight is influenced by energy intake (from food) and energy expenditure (the energy used for basal metabolism such as keeping the heart beating and for physical activity). If a person regularly consumes more energy than they use up, they will start to gain weight and eventually become overweight or obese. If a person regularly consumes less energy than they use up they will lose weight. Extra energy is stored in the body as fat.

Are there health risks associated with being obese?

Yes! Obesity is the most important dietary factor in chronic diseases such as cancer, cardiovascular disease and type 2 diabetes. Obesity causes a number of metabolic disturbances that can adversely affect an individual's chance of suffering from other chronic diseases later in life. People who are overweight or obese are more likely to suffer from coronary heart disease, type 2 diabetes, gallstones, osteoarthritis, high blood pressure and some types of cancer. Women are more likely to have complications during and after pregnancy.

How many people in Europe are obese?

Researchers have tried to estimate the number of obese people in the EU countries, and their findings are startling. Within the 15 countries that made up the EU in 2002, an estimated 18% of men and 21% of women were obese. This varied within countries with Austria topping the list with an alarming 30% of men and 26% of women being obese. Conversely, the rates in the Scandinavian countries are low (approximately 15% of men and 16% women). Projecting current trends in the increasing numbers of Europeans who are becoming obese to 2025, a staggering 30% of men and 34% of women will be obese unless something is done to reverse the trends.

How much is this costing the EU each year?

The health implications of obesity cost the European economy an estimated €32.8 billion in 2002. This figure includes the costs associated with treating the complications of obesity and lost productivity from days taken off sick. Unless something is done to stop the predicted increase in the prevalence of obesity in Europe, the consequences for both health and the economy are likely to be severe. Obesity and its related complications may end up costing the EU up to 6% of its health care budget in 2025.

What can be done to halt this alarming rise in obesity?

There is no easy solution to this problem as obesity is such a complicated disease. There are a number of different factors that affect an individual's risk of becoming obese and so it is essential that a number of different angles are taken when tackling the problem. Yet the potential for small lifestyle changes to have an effect must not be underestimated. In most cases, obesity is the result of very gradual adult-onset weight gain (estimated as approximately 1g/d). Indeed, one researcher has commented that 90% of obesity could be abolished by walking an extra 2000 steps a day and reducing intake by 100kcals a day.

Why don't we all just eat a bit less and exercise a bit more?

People have to be motivated to make lifestyle changes and if they do not appreciate that there is a problem, they are often not willing to cut down on food intake or exercise more, as they perceive this to have a negative effect on their quality of life. Therefore it is essential that other ways to help people change their diet and lifestyle are explored. In particular, we need to look for ways in which we can prevent the negative health effects associated with obesity such as high blood cholesterol levels.

Are there any ways to help people change their diet and lifestyle?

One option is to alter the nutrient composition of foods that are commonly consumed so that they are 'healthier'. This way, people do not have to make radical changes to their lives and they can continue eating the foods that they are used to. There a number of ways that this can be achieved. It is possible to alter the fatty acid profile of foods such as meat or milk by changing the composition of the animal feed. This way, the amount of saturated fatty acids is reduced and the amount of mono- and polyunsaturated fatty acids, which are better for heart health, are increased. An alternative approach is to add omega 3 fatty acids to foods such as yoghurt at the final stage of the production process as this type of fatty acid can reduce the risk of coronary heart disease. Researchers are even looking into ways in which plants, such as oil seed rape, can be modified to produce the long chain omega 3 fatty acids that are currently only available from algal or oily fish sources.

Are modified foods very expensive to manufacture?

They can be. There are increased costs associated with producing these sorts of products for many reasons. Firstly, the producers have to keep the food separate from the conventional product as it is important that its beneficial property is maintained. This can be a costly process if new equipment needs to be purchased. Also, at the moment so few people are currently buying these modified foods (referred to as functional foods) that they are often made on much smaller-scales. Because of this, the production costs are not spread over a large consumer base as they are for more popular foods and so instead they make up a sizeable proportion of the retail price. Also, in many cases, these products require special ingredients that can be quite expensive.

So if we have to pay extra for these foods, can we be sure that they will have a positive effect?

Lower fat polyunsaturated spreads were one of the first of these 'functional' foods available and their introduction had a massive impact on the types and amounts of the various fatty acids that were consumed in the diet. Saturated fatty acid intake fell and polyunsaturated fatty acid intake increased.

Estimates have been made to predict the impact that enhancing the omega 3 fatty acid content of meat, milk and eggs have on fatty acid intakes in Europe given current consumption patterns. In the UK, for example, intakes of the beneficial long chain omega 3 fatty acids are in the region of 244mg per day. However as oily fish contribute about 131mg per day of this total, and as only about 27% of the UK population consume any oily fish, the average intake for the vast majority of the population will be only about 113mg per day. For those who consume little or no fish the intake will be as low as 46mg per day – nowhere near the UK recommended 450mg per day. Enhancing the omega 3 fatty acid content of meat, milk and eggs could increase intakes from non-fish sources by up to 200mg per day.

Thus these foods, if eaten in the context of a healthy, balanced diet and lifestyle, would be expected to have a positive effect on health.

Can anything be done to increase the consumption of these foods across Europe?

The main factor that determines purchasing patterns are price and taste. The health benefits of a product are usually a lower priority. As obesity and coronary heart disease are more prevalent amongst lower income groups it's important that the price of healthier options is within their reach, so that those who will benefit most from the foods can afford to buy them. Various options have been considered by researchers in order to achieve this. For example, one option is for the EU to introduce subsidies on these 'healthier' foods that cover the extra ingredient costs. This way, the food manufacturers will be able to price their products in line with the conventionally produced foods. Or alternatively the EU could mandate that all animal products available are healthier thus making the market for meats, milks and eggs a much more level paying field. In this instance the public would be picking up the bill for the increased costs, but in this scenario there would be no cheaper, less healthy option. The final option suggested is that those people identified to be most at risk of obesity and other diseases could be encouraged to switch to the healthier brands by targeting subsidies directly at them. In this case, it is those who would benefit most from a dietary intervention who are receiving an economic incentive to modify their diets.

Have initiatives like this been carried out before and have they been successful?

Many functional foods are available in supermarkets across Europe but are often sold at higher prices because of the reasons discussed above. In order to encourage those at an increased risk of cardiovascular disease, private heath insurance companies in France and the Netherlands have devised incentives for their members to buy phytosterol spreads. These spreads have been shown to reduce the levels of LDL cholesterol (the bad type) in the blood and thus reduce the risk of developing heart disease. In this instance, a targeted portion of the population are being encouraged to switch to a healthier spread in the hope that it will reduce the chances of them later having to receive treatment for heart problems.

Appendix 3: *Lipgene* Publications

Human Nutrition Publications

Balwierz A, Czech U, Polus A ***et al.*** (2008) Human adipose tissue stromal vascular fraction cells differentiate depending on distinct types of media. ***Cell Proliferation*** **41**: 441-459.

Balwierz A, Czech U, Grzybowska-Gałuszka J ***et al.*** (2006) Proangiogenne właściwości komórek progenitorowych izolowanych z tkanki tłuszczowej ludzkiej. Prioangiogenic activity of progenitor cells isolated from human adipose tissue. Sympozjum Naukowe pt. "Perspektywy Kardiologii Zapobiegawczej w Polsce", Warszwa, Polska, 16 grudnia 2006. ***Czynniki Ryzyka*** **4 (50)**: 63

Balwierz A, Czech U, Grzybowska-Gałuszka J ***et al.*** (2006) Proangiogenne właściwości komórek progenitorowych izolowanych z tkanki tłuszczowej ludzkiej. Prioangiogenic activity of progenitor cells isolated from human adipose tissue. XI Sympozjum Sekcji Kardiologii Eksperymentalnej Polskiego Towarzystwa Kardiologicznego Komisji Krążenia i Oddychania Komitetu Nauk Fizjologicznych PAN Komisji Kardiologii Komitetu Patofizjologii Klinicznej PAN, Krzyżowa-Wrocław, Polska, 19-21. październi ***Kardiologia Polska***. **64 (10)** S6: 633-634

Balwierz A, Czech U, Grzybowska-Gałuszka J ***et al.*** (2005) The influence of fatty acids on differentiation of the adipose tissue stromal vascular fraction (SVF) cells. The Third Annual Meeting of the European Stem Cell Therapeutics Excellence Centre (STEC)and The Second Meeting of the Polish Stem Cell Excellence Network, Cracow, Poland, 6-9 October 2005 W: ***Proceedings of Stem Cell Research***: **53**

Balwierz A, Czech U, Polus A ***et al.*** (2007) Differentiation of human adipose tissue progenitors to adipocytes or endothelial cells using different culture conditions. 42 Meeting of the Polish Biochemical Society, Szczecin, Polska, 18-21 September 2007. ***Acta Biochimica Polonica*** **54 (S4):** 31

Balwierz A, Czech U, Polus A ***et al.*** (2007) Proangiogenic activity of progenitor cells isolated from human adipose tissue. 10th European Nutrition Conference, Paris, France, 10-13 July 2007. ***Annals of Nutrition & Metabolism*** **51 (S1):** 367

Balwierz A, Czech U, Polus A ***et al.*** (2007) Proangiogenic activity of progenitor cells isolated from human adipose tissue. EUROBIOTECH. Biotechnology in Agriculture, Polska, Kraków, 25-27 April 2007. ***Acta Biochimica Polonica*** **54 (S1):** 6

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Balwierz A, Czech U, Grzybowska-Gałuszka J ***et al.*** (2006) Proangiogenne właściwości komórek progenitorowych izolowanych z tkanki tłuszczowej ludzkiej. Prioangiogenic activity of progenitor cells isolated from human adipose tissue. XI Sympozjum Sekcji Kardiologii Eksperymentalnej Polskiego Towarzystwa Kardiologicznego Komisji Krążenia i Oddychania Komitetu Nauk Fizjologicznych PAN Komisji Kardiologii Komitetu Patofizjologii Klinicznej PAN, Krzyżowa-Wrocław, Polska, 19-21 październi. ***Kardiologia Polska*** **64 (10)** S6: 633-634

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