Overview of presentations given at the LIPGENE conference (The ticking time bomb: the metabolic syndrome), held in London in December 2004.

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Introduction

The prevalence of obesity, overweight and type 2 diabetes are all increasing across Europe. Associated with obesity and type 2 diabetes are a range of metabolic disturbances and risk factors for cardiovascular disease (CVD) (i.e. coronary heart disease (CHD) and stroke). When these metabolic disturbances cluster together, they are known as the metabolic syndrome.

The metabolic syndrome (also known as syndrome X and the insulin resistance syndrome) is the term used to describe a cluster of related characteristics associated with cardiovascular disease (CVD) and type 2 diabetes, including obesity (especially central obesity), hypertension (raised blood pressure), abnormal blood lipids (dyslipidaemia), insulin resistance and high blood glucose concentrations. The incidence of the metabolic syndrome increases with age, as life expectancy is increasing across Europe the prevalence of the metabolic syndrome is likely to increase further. This has implications for both healthcare and social welfare costs, as well as for the European economy overall. Currently it is estimated that between 10 and 35% of the middle aged and elderly population in Europe have metabolic syndrome.

As the prevalence of the metabolic syndrome is increasing, strategies to prevent further rise in the metabolic syndrome need to be implemented urgently. Dietary strategies coupled with increased physical activity appear to be effective; combined there seems to be a synergistic effect, which may be more effective than drug therapy (see Buttriss 2005 for an overview). Research is underway to determine which strategies may be most effective. LIPGENE is one such research project which is currently investigating this. The LIPGENE project, entitled Diet, genomics and the metabolic syndrome: an integrated nutrition, agro-food, social and economic analysis, is an EU Sixth Framework Programme Integrated Project (contract FOOD-CT-2003-505944), conducted by a consortium of 25 research centres across 14 European countries. It is investigating the relationship between diet and genetics in relation to the metabolic syndrome. The project began in February 2004 and will run for 5 years.

More details about the project can be found at: www.lipgene.tcd.ie or www.nutrition.org.uk/lipgene, but briefly, the LIPGENE project comprises six work packages which aim to:

- Examine how variation in the composition of dietary fats interacts with common human genetic variations to influence the development of the metabolic syndrome
- Reconstitute, in a plant oil, a sustainable source of long chain n-3 polyunsaturated fatty acids (the specific fatty acids from fish oil), that are known to play a role in reducing the risk of the metabolic syndrome
Develop a protocol for feeding dairy cows, which changes the composition of milk fat to one with less saturates, and more monounsaturates and without a substantial increase in trans fatty acids (i.e. to one with a more favourable fatty acid profile)

Explore consumer awareness about the metabolic syndrome and the potential health risks associated with it, as well as gauging consumers’ attitudes to newly developed agro-food technologies

Examine the economic barriers to introducing new agro-food technologies and the cost of dietary versus pharmaceutical approaches to managing the metabolic syndrome.

A further component of the project will be the demonstration of how technologically modified food products can be developed. These products will also be tested for consumer acceptance. One of the six work packages is devoted to dissemination, with the objective of increasing awareness and understanding of the need to integrate diet and genetics in tackling the metabolic syndrome, the potential of the agro-food technologies to combat the metabolic syndrome, and consumer and economic perspectives of the disease and its treatment. As part of the dissemination programme and in collaboration with the Nutrition Society, a conference, The Ticking Time Bomb: The Metabolic Syndrome, was held in London in December 2004. A brief overview of each of the talks can be found below. The full proceedings from the conference will be published in Proceedings of the Nutrition Society in August 2005. In addition, the papers can be purchased as a separately bound set, in a special issue (click here to for the order form).

Ms Rosanna D’Amario of the Food Quality and Safety Priority, EU Commission, Brussels, started the day’s proceedings by providing an introduction to the LIPGENE project, describing the EU’s role in funding research and highlighting related EU-funded research. Professor Christine Williams (Hugh Sinclair Unit of Human Nutrition, University of Reading) then summarised current understanding about the metabolic syndrome and its implications (Shaw et al, 2005). Following on, Dr James Fry of LMC International spoke from an economic perspective about the prevalence and costs of obesity (Fry et al, 2005) and Dr Barbara Stewart-Knox, of the Northern Ireland Centre for Food and Health, University of Ulster presented emerging research about the psychological underpinnings of obesity and metabolic syndrome (Stewart-Knox 2005) Dr Helen Roche (a coordinator of LIPGENE) of the Nutrigenomics Research group at the Institute of Molecular Medicine, Trinity College Dublin, spoke about interactions of diet and genetics in the aetiology of the metabolic syndrome (Roche 2005). Next, Dr Paulus Verschuren of the Unilever Health Institute, The Netherlands discussed the potential of modern fat technology to positively influence heart health (Upritchard et al, 2005). Following this, Professor Johnathan Napier of the Crop Performance and Improvement Division, Rothamsted Research, spoke on the production of very long chain polyunsaturated fatty acids in transgenic plants (Napier et al, 2005). Continuing the theme of agro-foods, Professor Ian Givens of the Nutritional Sciences Research Unit, University of Reading presented on the topic of animal science and opportunities for the future. Finally, to sum up, Dr Sinéad McCarthy of the Department of Clinical Medicine, Trinity College Dublin, presented a paper entitled ‘LIPGENE: what, how, why?’.
The metabolic syndrome and its implications

As mentioned above, the metabolic syndrome is characterised by insulin resistance, hyperglycaemia, (central) obesity, hypertension and dyslipidaemia (an abnormal blood lipid profile often referred to as the atherogenic lipoprotein phenotype) (see table 1). It is associated with an increased risk of CVD (2-3 fold) and type 2 diabetes (5-6 fold). Other characteristics of the metabolic syndrome may also include abnormal vascular function as well as pro-inflammatory conditions (such as microalbuminuria and hypercoagulability). Although the prevalence of obesity increases with age, the metabolic syndrome has now been identified in overweight children. There is particular concern about the consequences of the increasing prevalence of the metabolic syndrome in young people as studies suggest that many children with the metabolic syndrome will go on to develop type 2 diabetes (Weiss et al, 2004).

It is not known whether insulin resistance is a primary cause of the metabolic syndrome or a consequence of obesity. Both obesity and insulin resistance are key features of the metabolic syndrome, and due to their close relationship, it is difficult to disentangle their individual effects and determine which is the key trigger of the metabolic syndrome. A recent prospective study suggests that obesity precedes the metabolic syndrome (Palaniappan et al, 2004), but more research is urgently required to confirm or refute this finding. It is as yet unclear whether this finding is linked to the effects of different patterns of fat distribution e.g. centrally distributed around the abdomen compared with deposition on the hips and thighs. Therefore, more research is also required to determine the pathophysiology of the metabolic syndrome and insulin resistance.

Table 1. Features of the dyslipidaemia associated with the metabolic syndrome

<table>
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<tr>
<td>Raised fasting triacylglyceride (TAG) concentrations</td>
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<tr>
<td>Normal LDL cholesterol concentrations. Increased proportion of small</td>
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<tr>
<td>dense LDL cholesterol</td>
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<tr>
<td>Reduced HDL cholesterol concentrations with an increased proportion of</td>
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<td>large buoyant HDL cholesterol particles</td>
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<tr>
<td>Impaired post-prandial lipaemia (especially the clearance of TAG)</td>
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There is emerging evidence that adipose (fat) tissue and specific adipose stores may play an important role in the development of the metabolic syndrome. Adipocytes (fat cells) secrete a number of bioactive compounds into the circulation, including TNFα, IL-6, leptin and adiponectin, all of which have been implicated in the development of insulin resistance. In addition, the hydrolysis of adipocyte fat stores leads to the release of non-esterified fatty acids (NEFA) into the circulation. Elevated concentrations of NEFA themselves are associated with disturbances of lipid and glucose metabolism (Nugent 2004).

Disturbances in insulin homeostasis and insulin resistance, as seen in the metabolic syndrome, lead to short-term compensatory hyperinsulinaemia (additional insulin is produced by and secreted from the pancreas). As such a response can not be maintained, hyperglycaemia ultimately results. Hyperinsulinaemia can increase sympathetic nervous system activity and lead to hypertension, another key feature of the metabolic syndrome. In addition, raised fasting TAG concentrations observed in the metabolic syndrome
(see table 1) may also lead to hypertension and endothelial dysfunction (i.e. impaired vascular function) in individuals with insulin resistance.

There is no universal definition of the metabolic syndrome; this is urgently required in order to determine its true global prevalence in Europe and further afield, and to aid effective clinical diagnosis, policy development and the establishment of prevention programmes. At present, the numerous definitions of the metabolic syndrome vary considerably: most definitions include obesity or central obesity, hypertension and dyslipidaemia, but not all include insulin resistance. Therefore, estimations of the prevalence of the metabolic syndrome vary according to the definition used. Additionally, cut-off points for various parameters which define the metabolic syndrome need to be rigorously tested to ensure that they are appropriate. Individuals of African or Asian origin, resident in the Europe and North America, are more susceptible to the metabolic syndrome than individuals of European origin, and cut-off points for measurements (e.g. BMI, waist circumference or blood lipids) may not be appropriate and thus underestimate the true prevalence of the metabolic syndrome in some sub-groups of the population.

The true prevalence of the metabolic syndrome in adults aged over 65 years and the consequences of the metabolic syndrome also need to be determined. Life-expectancy is increasing across Europe, and the proportion of the population aged over 65 years is set to increase. It is thus likely that the metabolic syndrome will have a large impact on morbidity and health care costs amongst middle aged and elderly individuals. It is worth noting that the presence of metabolic syndrome in women is associated with a greater risk of CVD than for men. The reasons for this association need to be determined along with the excess risk.

At present the awareness of the metabolic syndrome amongst health professionals and the general public is low, and public health campaigns may be required to increase awareness. It is extremely important to rectify this situation to help ensure that individuals with metabolic syndrome are correctly diagnosed and treated, as the consequences of the metabolic syndrome can be severe. The metabolic syndrome is also associated with a number of other morbidities including eye disease, nerve damage and kidney disease, all of which impact on the overall quality of life and costs of health care (see below).

Changes in diet may have an impact on the prevalence of the metabolic syndrome, and more research is urgently required in this area. One area of particular interest is the impact of dietary fat composition on markers of the metabolic syndrome, although few human studies have been conducted in this area. Research conducted to date suggest that diets rich in monounsaturated fatty acids (MUFA) may reduce the adverse effects of high intakes of saturated fatty acids (SFA) on insulin sensitivity (Vessby et al, 2001), provided total dietary fat intake is not high (a benefit was seen in diets providing less than 37% of energy as fat). Some interest has also been expressed in the potential impact of dietary n-3 polyunsaturated fatty acids (see Buttriss 2005 for an overview). LIPGENE will study the effect of dietary fat quality, and of diet sensitive genotypes, on insulin sensitivity and risk markers associated with the metabolic syndrome. In addition, the impact of diet on such markers will be investigated in individuals with diet sensitive genotypes. Central to the research programme is a human intervention study in 8 European countries that will study the impact of changes in dietary fat composition on characteristics of the metabolic syndrome and interactions of these with common genetic variations.
Economics of the metabolic syndrome: the prevalence and costs of obesity

Associated with an increased prevalence of type 2 diabetes, obesity and the metabolic syndrome are the increased healthcare (direct) costs required to treat the adverse health implications. Losses to the economy (indirect costs) through reduced productivity at work and increased absenteeism caused by related illnesses should also be recognised as a burden to the European economy. As mentioned above, obesity is one of a number of components of the metabolic syndrome, the true global prevalence of which is unknown as there is no universally accepted definition of the metabolic syndrome. This lack of definition makes it hard to estimate the true costs.

Using data published by the UK National Audit Office (NAO) on the estimated direct and indirect costs associated with obesity in England for the year 1998 (Comptroller and Auditor General 2001), along with data on the prevalence of obesity (estimated to lie between 11 and 38% in 2002: OECD, 2004), costs associated with obesity across the then 15 EU member states have been estimated (minus Luxembourg for which there is no available data) for the year 2002. Using extrapolated data, it is estimated that in 2002 the direct and indirect costs of obesity within the EU were in the region of €32.8 billion. These estimates are conservative, and as obesity is only one component of the metabolic syndrome, the costs of the metabolic syndrome are likely to be higher.

Diet may play an important role in tackling the exploding prevalence of both obesity and the metabolic syndrome, and the provision of novel foods with an altered nutrient profile may provide a useful tool to help reduce the incidence of the metabolic symptoms and associated complications. Producing and providing foods with an altered nutritional composition and a healthier nutrient profile is likely to cost more than producing the standard version of that food. There are three main reasons for this: higher costs of ingredients; increased costs associated with a lack of/smaller operational scale of production; and identity preservation costs, required to preserve the composition of the product and prevent cross-contamination with other less valuable products, which would dilute the value of the product. Therefore, there is a necessary price premium associated with novel foods providing additional health benefits, e.g. stanol/sterol enriched margarines or n-3 fatty acid enriched hens’ eggs. Foods with appropriately modified fatty acid compositions (e.g. milk and meat) could potentially help reduce the health and economic burden associated with the metabolic syndrome due to their positive influence on blood lipids. In the long term they may also have a beneficial impact on the prevalence of the metabolic syndrome.

Owing to the additional costs associated with producing and marketing novel foods, it can be assumed that their share of the market will be lower than that of the standard version of that particular food, as target consumers may be unwilling to pay the premium price. To encourage consumption, the costs associated with their production (i.e. the associated premium) may need to be abolished or at the very least minimised so that there is little price difference between the standard and novel versions of a food product, to ensure that the product is purchased. One way of achieving this is through subsidisation, to help ensure that novel food products with genuine health benefits command a greater share of the market for that particular food category.
Dr Fry’s economic analysis hypothesises that the cost of subsidisation, to enable the extra costs associated with novel food production to be brought down to zero, can be compared with the direct and indirect costs associated with obesity. He has estimated that the extra costs associated with the production of meats and eggs with favourable fatty acid profiles and modified fat spreads across the EU-15, based on their market share in 2002, are estimated to be in the region of €10 billion; which is approximately a third of the total cost of obesity in 2002 (€32.8 billion). As the provision of foods with an altered fatty acid or nutrient profile has the potential to prevent and treat the metabolic syndrome and its associated complications, the provision of these foods in the long-term also has the potential to alleviate the economic burden associated with these disorders.

These issues will be discussed in more detail at two LIPGENE workshops, the first of which will take place on May 25th 2005 in Brussels. Information from these workshops will appear on the LIPGENE website in due course.

Psychological underpinnings of metabolic syndrome

Certain psychological traits and behaviours, psychological distress, emotional stress and adverse social circumstances appear to play a role in the development of the metabolic syndrome. Certain personality traits may also confer a greater risk of developing the metabolic syndrome. For example, competitiveness, impatience, hostility and time urgency (collectively termed type A pattern coronary prone behaviour) are associated with characteristics of the metabolic syndrome. Anger, which is one aspect of hostility, is associated with elevated blood pressure and may itself also increase the risk of metabolic syndrome. Associations have been observed between anger and risk of metabolic syndrome (Raikkonen et al, 2002) and also between visceral adiposity (central obesity) and both hostility and anger in post-menopausal women (Raikkonen et al, 1999). Both acute and chronic stress are associated with an increased risk of CHD and type 2 diabetes. Chronic stress has been associated with both visceral obesity and metabolic syndrome (Steptoe & Marmot 2003) and it may be that stress can trigger or exacerbate the metabolic syndrome. Anxiety and clinical depression may also be associated with the metabolic syndrome and it has been suggested that such associations are stronger amongst women than men, suggesting that there may be gender specific differences in the aetiology of the condition.

Prevalence of the metabolic syndrome is highest amongst socially and economically disadvantaged groups. The association between deprived socio-economic status, poor health and the metabolic syndrome may be caused by hostility and anger associated with disadvantaged social circumstances and may reflect a greater degree of stress experienced by the socially disadvantaged (Bjorntrop 2000; Brunner et al, 1997). This may in turn impact on lifestyle factors associated with increased risk of the metabolic syndrome, e.g. poor diets and inactivity. A lack of social support or social isolation are also associated with metabolic syndrome regardless of social class, suggesting that support may offer some degree of protection from the influence of deprived circumstance on risk. The impact of social exclusion on risk of developing the metabolic syndrome is greater in men than women, again implying gender differences in the causation of metabolic syndrome. A lack of social support and coping skills may exacerbate stress. Whether adverse social circumstances and psychological factors are the cause or effect of ill health has yet to be determined, however data suggest a
positive attitude towards life and the forging of intimate relationships may help reduce the risk of developing the metabolic syndrome.

The mechanisms whereby different psychological and physiological factors act to bring about the metabolic syndrome are not known. Two mechanisms have been proposed, the first, involving the hormone cortisol and the second, the neurotransmitter serotonin. It has been hypothesised that stress brings about the metabolic syndrome through the action of cortisol on acute inflammatory responses (inflammation is associated with an increased risk of CVD and type 2 diabetes), and high salivary cortisol concentrations are associated with abdominal obesity, hypertension and dyslipidaemia, providing support for this hypothesis. With regards to serotonin, associations between abdominal obesity, metabolic syndrome and depression (associated with impaired serotonin activity) have been observed. Furthermore, the long-term use of selective serotonin re-uptake inhibitor (SSRI) drugs in the treatment of depression is associated with an increased risk of metabolic syndrome (Almeras et al, 2004; Vieta 2004) and evidence from animal and human studies suggests that increased serotonin turnover is associated with the development of obesity. Serotonin may also be associated with raised TAG concentrations, hypertension and insulin resistance; all manifestations of the metabolic syndrome.

In conclusion, stress and other adverse psycho-social factors may play a role in the metabolic syndrome. Metabolic changes associated with stress may be cortisol and/or serotonin mediated. Such associations between psycho-social circumstances and the metabolic syndrome provide a new avenue for the development of prevention strategies and treatments for metabolic syndrome (e.g. stress reduction). It is important, therefore, to determine the impact of psycho-social factors in the aetiology of the metabolic syndrome.

The LIPGENE project will investigate the association between psychological and behavioural traits, social and demographic factors, lifestyle and cultural factors and the metabolic syndrome. A large population study (n=1500) of individuals aged over 50 years with the metabolic syndrome will help to determine the relative contributions of the above factors to the aetiology of the condition.

**Metabolic syndrome: the role of diet and genetics**

Genetics, the environment and their interactions play a role in the aetiology of the metabolic syndrome. Nutrition is one such environmental factor and nutrient-gene interactions may play a significant role in the development of metabolic syndrome. Understanding such interactions will provide important insight into the metabolic syndrome and such information could be used to manage the disorder.

The metabolic syndrome is a progressive disorder, whereby the liver, skeletal muscle and adipose tissue become resistant to the actions of insulin. For this reason it is difficult to determine the genetic components of the metabolic syndrome. Genes which are influenced by environmental (including nutrient) interactions may include those involved in pancreatic β-cell function (the site of insulin production), glucose metabolism, insulin action, blood pressure regulation and lipoprotein and lipid metabolism. Indeed evidence suggests that there is a genetic influence on fasting glucose concentrations and β-cell function (Mills et al, 2004) and that fasting glucose, insulin, triacylglyceride (TAG) and HDL-cholesterol concentrations are influenced by
hereditary factors (Freeman et al, 2002). Furthermore, certain ethnic groups are at a higher risk of the metabolic syndrome than others. Once the molecular basis of the metabolic syndrome has been determined it will be possible to ascertain the effect of gene-nutrient interactions on the metabolic syndrome.

Although both genetic and environmental conditions contribute to the development and progression of the metabolic syndrome; their relative contributions will vary for each component of the disorder. It is also hard to disentangle the effects of one from the other. There is evidence that genetics has a greater influence on glucose intolerance, obesity and HDL cholesterol than environmental factors, whilst environmental factors appear to contribute greatly towards the variability in fasting insulin and TAG concentrations as well as blood pressure (Poulsen et al, 2001). It is likely that recent changes in dietary habits and physical activity patterns, coupled with genetic susceptibility, have played a large role in the increased prevalence of the metabolic syndrome that we are seeing today.

Attempts have been made to identify genes involved in the development and progression of the metabolic syndrome. However, this has proved difficult, the metabolic syndrome comprises of a cluster of risk factors and so a large number of genes have the potential to be involved in its development. Genes involved in the development of type 2 diabetes have been identified. For example, having the common pro12Ala polymorphism of the peroxisome proliferator activator receptor (PPAR)γ gene is associated with a significantly increased risk of type 2 diabetes (Altshuler et al, 2000). It has also been suggested that variants of this gene may play a role in the development of the metabolic syndrome as the PPARγ gene encodes for a transcription factor which regulates adipogenesis (fat cell formation) and lipid and glucose metabolism (Barak et al, 1999; Barroso et al, 1999). Another gene that has been identified is Calpain 10 (CAPN10), where an adenine (A) to guanine (G) polymorphism (whereby an adenine base is replaced with a guanine base) on intron 3 of the gene is associated with an increased risk of type 2 diabetes (Horikawa et al, 2004). Additionally, modest associations between CAPN10 polymorphisms and phenotypes (sets of characteristics) associated with the metabolic syndrome have been made (Florez et al, 2003).

The fatty acid composition of the diet can impact on the risk of the metabolic syndrome through its influence on insulin action, lipid metabolism and lipoprotein concentrations. As components of diet can interact with our genotypes. Associations between habitual dietary fatty acid intake, polymorphisms of the PPARγ gene and fasting insulin and glucose concentrations, BMI, and visceral adiposity (central obesity) have all been reported (Luan et al, 2001; Robitaille et al, 2003), but further studies are required as associations have not been equivocal and discrepancies have been reported. Additionally, differences in gene polymorphisms have been shown to influence an individual’s responsiveness to dietary and exercise interventions; again this has been demonstrated with polymorphisms of the PPARγ gene (Franks et al, 2004).

The LIPGENE project will examine how variations in the composition of fat in the diet interact with common genetic variations in humans to influence the development of the metabolic syndrome. This research will involve the analysis of existing dietary, biochemical, clinical and genetic data from 13,000 subjects and the initiation of a comprehensive multi-centre human feeding intervention study (see above).
Modern fat technology

Currently, it is recommended that individuals reduce intakes of SFA. Current dietary recommendations in terms of dietary fats are detailed below (table 2). Changing the fatty acid content of the diet can influence blood lipid concentrations and thus impact on the risk of developing CHD and the metabolic syndrome: diets rich in SFA are associated with increased total, and LDL, cholesterol concentrations. Substituting SFA with other dietary fats, therefore, tends to have desirable consequences on blood lipids. For example, substituting SFA with an equal amount of MUFA or PUFA reduces both total and LDL cholesterol concentrations without affecting HDL cholesterol concentrations. Substituting SFA with the long chain n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic (DHA) additionally reduces triacylglyceride (TAG) concentrations. However, substituting SFA with trans fatty acids increases TAG concentrations and reduces HDL cholesterol.

Table 2: Current dietary fat recommendations

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<thead>
<tr>
<th>Type of fat</th>
<th>Dietary Recommendation</th>
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<tr>
<td>Total fat</td>
<td>30-35% energy</td>
</tr>
<tr>
<td>Saturated fat</td>
<td>10% energy</td>
</tr>
<tr>
<td>Polyunsaturated fatty acids</td>
<td>4-10% energy</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>4-8% energy</td>
</tr>
<tr>
<td>α-linolenic acid (LNA)</td>
<td>0.5-1% energy or 2g/day</td>
</tr>
<tr>
<td>EPA and DHA</td>
<td>200-500mg/day</td>
</tr>
<tr>
<td>Monounsaturated fatty acids</td>
<td>30-35% of energy from fat</td>
</tr>
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Margarines and fat spreads are often consumed on a daily basis and therefore they have the potential to influence intakes of fatty acids and nutrients. Indeed, margarines and reduced-fat spreads contribute vitamins A, D and E to the diet and can help reduce intakes of SFA and increase intakes of PUFA, if used in place of fats rich in SFA. Over time, margarines and reduced-fat spreads have embraced advances in knowledge and technology so that they reflect current dietary recommendations with regards to cardiovascular health, and provide a vehicle to deliver improved nutrition without the need for concerted effort on behalf of the consumer.

Margarine, produced originally from beef fat and milk as a substitute for butter, was invented in 1869 by Hippolyte Mège-Mouriès. Over time, as knowledge on fatty acids and health has advanced, the composition of margarine has been modified to incorporate different fats (as they became available) and take account of new technologies. In 1960, the first margarine high in PUFA (50-55%) and low in SFA was produced. Conforming to the then current recommendations concerning dietary fatty acids and coronary health, this margarine was marketed as a substitute for butter and was originally only available on prescription. During the 1970s, reduced-fat margarines (i.e. reduced-fat spreads) were developed, providing less energy and 50% less fat than standard margarines. In order to achieve this reduced fat content, these spreads contained a greater proportion of water, whilst at the same time the proportion of fatty acids as PUFA
remained high and SFA low. In order that consumers accepted such products, these reduced fat spreads needed to provide the same characteristics as standard margarines and a number of issues concerning their production first needed to be overcome.

Vegetable oils, used to make margarines, are not solid at room temperature. Therefore, a number of methods are used to harden fats and produce margarines. One such method is the hydrogenation of oils. This hydrogenation process leads to the production of trans fatty acids (they also occur naturally in milk and dairy products, butter and in oils heated to high temperatures). In the 1990s, as evidence emerged that trans fatty acids have a detrimental effect on blood lipid profiles (i.e. increase total and LDL cholesterol and reduce HDL cholesterol), the margarine industry was challenged with removing trans fatty acids from spreads, whilst at the same time keeping the SFA content constant. Gram for gram trans fatty acids are worse than SFA, but intakes tend to be far lower. For example, in the UK, intakes of trans fatty acids are in the region of 1.2% of energy (well below the 2% energy target) compared to SFA, intakes of which are in the region of 13% (Henderson et al, 2003). Today, most margarine manufacturers in Europe have removed trans fatty acids from fat spreads and this means that, on average, margarine fat spreads contain about 2% of fats as trans fatty acids (FSA 2004), but to compensate their SFA content has risen slightly. Producing margarines without using hydrogenation and without increasing the SFA content has proved a challenge, but is possible. A number of different processes can be used, for example a process known as fractionation followed by interesterification or hydrogenation followed again by interesterification (Upritchard et al, 2005).

Research indicates that increased total PUFA intakes are associated with reduced incidence of CHD. n-6 fatty acids have been recognised to lower blood LDL-cholesterol and n-3 fatty acids (at least at high intakes) lower blood TAG concentrations. Currently, margarines providing both n-6 and n-3 fatty acids (in the form of linoleic and α-linolenic acids) are available: however, increasing the number of double bonds in the fat spread (as is the case here) increases its susceptibility to oxidation. Oxidation occurs during margarine manufacture and storage, and oxidation products have undesirable effects within the body. Therefore, oxidation needs to be minimised; this can be achieved by adding antioxidants and strictly controlling the manufacturing process. Additionally, margarines and fat spreads are available which contain plant stanols or sterols. These compounds are capable of reducing LDL cholesterol concentrations if consumed in sufficient quantities.

In the future, as knowledge of the links between nutrition and health advances, new opportunities for improving the nutrient profile of margarines and fat spreads will emerge. It may soon be possible to produce margarines containing EPA and/or DHA or we may be seeing fat spreads containing calcium or folic acid hitting the supermarket shelves.

In due course, as evidence emerges from the intervention studies, LIPGENE will include a demonstration project, co-ordinated by the Unilever Health Institute.
Production of long chain n-3 fatty acids in plants

The long chain n-3 PUFA in the form of EPA and DHA, have been shown to offer protection against CVD, particularly CHD. There is emerging evidence that they may also be important in relation to type 2 diabetes and the metabolic syndrome.

Mammals, including man, are not capable of synthesising n-3 PUFA de novo, and therefore they must be consumed preformed in the diet. The parent fatty acid of the n-3 series is α-linolenic acid ALNA, which is present in plant foods such as vegetable oils. ALNA can be metabolised (through elongation and desaturation) by mammals to produce EPA and subsequently DHA, but the capacity for this is limited. Therefore, the majority of long chain n-3 fatty acids are acquired through diet. Oily fish and fish liver oils are the predominant source of EPA and DHA, with very much smaller amounts being present in meat and eggs. However, there are concerns that stocks of certain fish are on the decline and will not sustainable in the long-term. As current intakes of EPA and DHA are less than desirable in many European populations and as advice is to increase intake of long chain n-3 PUFA in the diet, a sustainable source of these fatty acids is required. Alternative sources of long chain n-3 fatty acids include microbiological sources such as algae; another possible source is transgenic plants, which have been genetically engineered to synthesise EPA and/or DHA.

Fish are also not capable of synthesising n-3 fatty acids and acquire n-3 fatty acids through their diet, mainly through the consumption of micro-organisms (such as algae) or the consumption of smaller fish that have eaten these PUFA-synthesising micro-organisms themselves: n-3 PUFA pass up the food chain, from micro-organisms, to fish, to man. Farmed fish do not have the same direct access to a ready supply of these algae and smaller fish. Therefore, they must be provided with fish oils rich in long chain n-3 fatty acids as part of their diet; aquaculture (fish farming) is the main consumer of fish oils and this practice is unsustainable in the long term (Napier et al., 2005).

Higher plants can synthesis PUFA (predominantly the n-6 series fatty acids) but not long-chain PUFA with a carbon chain of 20 or more atoms. However, micro-organisms are capable of synthesising long chain n-3 PUFA efficiently, including EPA and DHA. The synthesis of long chain PUFA from a substrate fatty acid (e.g. LA or ALNA) involves the sequential insertion of double bonds and two-carbon elongation, using desaturase and elongase enzymes respectively. The possibility of higher plants producing EPA and DHA in storage lipids of their seeds is being investigated by Professor Napier and his colleagues. A transgenic plant that is capable of synthesising long chain n-3 PUFA would provide a cheap and sustainable source of EPA and DHA. In order for this to be possible, biosynthetic genes encoding for long chain n-3 PUFA biosynthesis would need to be inserted into a suitable oilseed crop through genetic engineering. Such a plant would be said to contain a ‘trait’ for long chain n-3 PUFA synthesis. Firstly, such genes need to be identified and their function characterised. Following this, these genes need to be reconstituted in the new host.

A number of genes encoding for desaturase and elongase enzymes have been identified (e.g. in fungi and algae). All the desaturases involved in PUFA biosynthesis have been identified and furthermore, many have been functionally characterised in non-native host species, such as yeast and transgenic plants, and observed to function efficiently. Elongases are not as easy to characterise, as four different and sequential
enzymes are involved in chain elongation. However, it has been suggested that just the first of these enzymes is required.

Transgenic plants have been used successfully to synthesise long-chain PUFA in the form of arachidonic acid (AA, a 20 carbon n-6 fatty acid) and EPA, from endogenous LA and ALNA respectively. The ratio of AA: EPA yielded in the leaves of the transgenic plant was not reflective if the n-6:n-3 fatty acid ratio of the substrates and the predominant fatty acid produced was AA (Qi et al, 2004). Successful attempts, such as this, form an important breakthrough in terms of achieving a sustainable source of long-chain PUFAs and highlight the feasibility of such work. PUFA biosynthesis needs to be replicated in transgenic oil seed crops, which yield high-quantities of fatty acids in their seeds. Research is underway in this area: transgenic linseed and tobacco plants have been produced which are capable of synthesising AA and EPA. Yields have been proportionally low (EPA yield in transgenic plants is in the region of 3% of total fatty acids) but significant non-the-less (Abbadi et al, 2004). More research is required in order to increase the yield of EPA.

Ultimately the production of DHA in transgenic plants is desired. EPA can be elongated and then desaturated to form DHA, therefore additional genes encoding for enzymes involved in this process need to be inserted into transgenic plants already capable of synthesising EPA. Such genes have been identified and functionally characterised, but a number of constraints apply to the production of long-chain n-3 fatty acid biosynthesis. These constraints need to be overcome and will form the focus of much anticipated research, including work being conducted within the LIPGENE project.

Animal Nutrition

Meat, milk and their products form an important part of the diet in Europe. Collectively these foods are the major source of protein, calcium and iron across the continent but they are also collectively the main source of fat, particularly SFA. There is strong evidence linking diets rich in SFA with an increased risk of CHD, the metabolic syndrome and a number of other chronic diseases, as diets rich in SFA are associated with increased total and LDL cholesterol concentrations and poor insulin sensitivity, impaired glucose tolerance, higher fasting plasma glucose and insulin concentrations.

However, not all SFA raise LDL cholesterol to the same extent: myristic acid (C14:0) (associated with milk fat) and palmitic acid (C16:0) (present in meat and milk products) are more hypercholesterolaemic than stearic acid (C18:0), which is considered to be a lipid neutral SFA. There is debate as to the effect of lauric acid (C12:0) (also associated with milk fat) on LDL cholesterol. As mentioned above, the substitution of SFA with MUFA and/or n-6 PUFA lowers total and LDL cholesterol (and thus reduces risk of CHD). n-6 PUFA exert greater cholesterol lowering effects than MUFA, but high PUFA diets (greater than 10% of dietary energy) are not recommended due to the potential for lipid oxidation. Diets providing EPA and DHA are associated with a reduced risk of CHD and can help to reduce fasting TAG concentrations (see Buttriss 2005 for an overview).

It is possible to alter the fatty acid composition of the diet by changing the composition of the food supply. This would require no direct action on the behalf of the consumer, yet has the potential to impact on the
prevalence of chronic disease. It is possible to alter the fatty acid composition of meat and milk through the manipulation of cattle’s diets; it would be desirable to reduce the SFA content of meat and milk whilst at the same time increasing MUFA and PUFA content slightly. A number of compounds which may be beneficial to long term health (e.g. bioactive peptides, phytochemicals, sphingolipids and butyrate) are also found in meat and milk (in particular) and therefore the manipulation of cattle’s diets should also aim to preserve or optimise levels of these compounds.

Typically, 70-75% of the fatty acids present in milk are SFA, 20-25% are MUFA, and the remainder are PUFA. It is possible to reduce the amount of C16-18 SFA and increase the amount of MUFA (as oleic acid) present in milk by increasing the supply of SFA of 18 carbon chain length, or greater, to the mammary gland, as stearic acid (C18:0) can be desaturated within it. This can be achieved by feeding cattle plant or oil seeds, including whole rape seed oil. Attempts have also been made to increase the PUFA content of ruminant meat using this same methodology, but PUFA are not synthesised to any extent in ruminant tissues. The possibility of increasing the amount of EPA and DHA in milk and beef has been explored using dietary fish oil supplementation. Whilst it is possible to increase the amount of PUFA in milk this way, it has proved difficult and further research is required to overcome this (see Givens 2005).

A milk with a modified fatty acid profile, so that it contains fewer SFA, has been shown to be able to help reduce total and LDL-cholesterol concentrations without affecting HDL cholesterol (Noakes et al., 1996). If such milks were to be adopted across Europe there would be the potential to reduce total and LDL cholesterol concentrations and hence disease risk without having to modify dairy product consumption to any great extent.

It is also possible to modify the fatty acid content of meat. Typically, 45-55% of fatty acids present in meat are SFA, 45-50% are MUFA, the remainder are PUFA. Most attempts to modify the fatty acid content of meat (beef in particular, but also lamb) have looked at ways in which to improve the PUFA:SFA (P:S) ratio of meat and enhance the n-3:n-6 ratio. Feeding cattle high forage based diets (ALNA is the major fatty acid in fresh grass) or PUFA rich oil supplements are the best ways of achieving this. Although most ALNA consumed by cattle will be hydrogenated in the rumen, a small proportion can escape this process, be absorbed and incorporated into tissue lipids. Unlike ruminant meat, the fatty acid profile of non-ruminant meat reflects dietary fatty acid composition. In pigs and poultry, there is little metabolism of ALNA to EPA and DHA, therefore fish oil needs to be provided in the diet in order to increase tissue EPA and DHA. Whilst this has been relatively effective, fish oil supplementation produces meat with a metallic taste, fish like flavour and reduced shelf life. Therefore, an alternative source of EPA and DHA is required that results in an acceptable product for the consumer.

Changes to feeding of animals would incur a cost and may require political and financial incentives as well as changes to the animal production industry to be successful. It is important that such modifications of fatty acid profile do not negate any of the beneficial properties associated with meat, milk and their products.

Next steps
The LIPGENE project will run until 2009. To receive periodical e-mail updates on the project, sign up at: www.nutrition.org.uk/lipgeneupdates
Full papers from the _LIPGENE_ conference (The ticking time bomb: the metabolic syndrome) held in December 2004 are due to be published in August 2005 in the *Proceedings of the Nutrition Society*. An order form for a compilation of the papers can be found at [www.nutrition.org.uk/lipgeneconference](http://www.nutrition.org.uk/lipgeneconference)

General information about diet and health can be found at [www.nutrition.org.uk](http://www.nutrition.org.uk), and this website also has summary information derived from a recent British Nutrition Task Force report on diet and cardiovascular disease (*Cardiovascular Disease: Diet, Nutrition and Emerging Risk Factors*). For more information on diet and CVD see [www.nutrition.org.uk/cvd](http://www.nutrition.org.uk/cvd)

In addition, a manuscript is in preparation for a book entitled _Improving the Fat Contents of Foods_, to be published by Woodhead Publishing Limited. Further information will be publicised closer to publication date. The book includes chapters by members of the _LIPGENE_ consortium.

**References:**


