The metabolic syndrome (MetS) is a very common disease associated with an increased risk of type 2 diabetes mellitus and cardiovascular disease. The diverse clinical characteristics of the MetS illustrate the complexity of the disease process, which involves several dysregulated metabolic pathways. Thus, multiple genetic targets must be involved in the pathogenesis and progression of the disease. Research indicates a major role for genetic susceptibility to the MetS. However, the human genome has not changed markedly in the last decade but the prevalence of the condition has increased exponentially, illustrating the importance of gene–environmental interactions. Dietary fat is an important environmental factor which can modify the development of the MetS. Genetic background can interact with habitual dietary fat composition, affecting predisposition to the MetS. Recent research indicates that currently ineffective therapeutic dietary recommendations may require a ‘personalised nutrition’ approach, wherein the genetic profile may determine the responsiveness of patients to specific dietary fatty acid interventions. Understanding the biological impact of gene–nutrient interactions will provide a key insight into the pathogenesis and progression of diet-related polygenic disorders, including the MetS. This review will explore the interactions between genetic background and dietary exposure/nutritional therapy.

The Metabolic Syndrome – Definition, Prevalence and Preventative Strategies

The metabolic syndrome (MetS) is a very common, multi-component condition characterised by insulin resistance, dyslipidaemia, abdominal obesity and hypertension that is associated with an increased risk of type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD) and atherosclerosis [1]. In 1970, Berson and Yallow [2] defined insulin resistance as ‘a state (of a cell, tissue, system or body) in which greater-than-normal amounts of insulin are required to elicit a quantitatively normal response’, resulting in an inability of insulin to provide normal glucose and lipid homeostasis. Reaven [3] hypothesised that insulin resistance, glucose intolerance and hyperinsulinaemia are the underlying components of the MetS or ‘syndrome X’, and increase the likelihood of other abnormalities occurring (dyslipidaemia, endothelial dysfunction, coagulation and inflammatory disorders and abnormal uric acid metabolism).

Insulin resistance is a key feature of obesity, the MetS, T2DM and CVD. The pathway that links obesity and insulin resistance with the MetS and T2DM represents a progressive phenotype (fig. 1) [4]. Surplus adipose tissue generates excessive metabolic stress (non-esterified fatty acids (NEFAs)) and pro-inflammatory adipocytokines (tumour necrosis factor-α, leptin, interleukin-6, angiotensinogen, plasminogen activator inhibitor type 1, etc.) that impede systemic responsiveness to insulin, resulting in impaired insulin action, compensatory hyperinsulinaemia and glucose intolerance [5–8]. Insulin resistance
leads to a variety of abnormalities in the liver, muscle and adipose tissue resulting in dyslipidaemia characterised by elevated NEFAs and triacylglycerol (TAG) concentrations and low high-density lipoprotein (HDL) levels. Individuals with a sensitive genotype and who are at increased risk of developing the MetS will be most susceptible to the impact of metabolic and pro-inflammatory stressors.

The World Health Organisation (WHO) criteria for defining the MetS focuses on risk for diabetes centred around impaired glucose tolerance (IGT), impaired fasting glucose or insulin resistance as measured by the hyperinsulinaemic euglycaemic clamp [9]. The International Diabetes Federation (IDF) definition focuses on central adiposity and the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel (ATP) III) [10] assigns no priority to any of the criteria. In a recent perspective review, Reaven [11] summarised the similarities and differences of the three definitions of the MetS (WHO, IDF and ATPIII) but categorically stated that the components clustered in the syndrome occur only in insulin-resistant people. This is supported by results from animal studies [12, 13], and from human metabolic studies including the Insulin Resistance Atherosclerosis Study [14–16].

As there is no universal definition of the MetS, determination of the true global prevalence of the disease varies. Using the NCEP definition, the prevalence of the MetS is estimated to be 25% of the general population with no gender differences but varying with genetic background [17]. The IDF definition tends towards higher prevalence rates [18, 19]. Interestingly the WHO and the ATPIII of the NCEP definitions of the MetS have been shown to correlate closely in identifying people at risk of CVD [20]. Recently, Sandhofer et al. [21] compared the three definitions (WHO, IDF and ATPIII) with respect to metabolic parameters and their ability to predict intima media thickness and plaque extent in the carotid arteries. They found an increased prevalence of the MetS using the IDF criteria (25.8% for men and 19.5% for women). They also found that subjects identified by the WHO criteria (18.7% men; 16.2% women) had higher fasting insulin levels and were more insulin-resistant according to a higher homeostasis model assessment for insulin resistance than the subjects identified according to the NCEP ATPIII (18.9% men; 17.0% women) and the IDF criteria. They concluded that since the incidence of insulin resistance decreases with lower visceral obesity, using lower cut-off values decreases the specificity to detect insulin resistance [21]. Ford et al. [22] have reported an increase in the prevalence of the MetS from 23.1% in the National Health and Nutrition Examination Survey III (1988–1994) to 26.7% in the National Health and Nutrition Examination Survey (1999–2000).

Whilst diet and/or poor nutritional status are not risk factors for the MetS, reducing body weight, through manipulation of diet, is a well-accepted therapy to reduce the incidence of T2DM and improve risk factors such as hyperlipidaemia and hypertension. However poor compliance means that this therapeutic approach is largely ineffective and the prevalence of obesity continues to rise. Therefore other strategies to attenuate the impact of insulin resistance in the presence of obesity are required. Recently attention has shifted to the concept of ‘personalised nutrition’ wherein genetic testing preluding to appropriate nutritional or dietetic advice may be advocated. The exploration of genotype and diet and their interactions will be paramount both in new preventative programmes and in determining the risk of complex polygenic diet-related diseases [23], of which the MetS is at the forefront.

This article will review the evidence in relation to the genetic components of the MetS and then explore the hypothesis that this may be modified by dietary factors, in particular dietary fatty acid composition. Genetic background can determine an individual’s responsiveness to altered dietary fatty acid composition, therefore dietary therapy to reduce the risk of the MetS may require a ‘personalised’ nutrition approach.
Genetic and Environmental Determinants of the Metabolic Syndrome

The current global epidemic in the incidence of the MetS and T2DM is an important illustration of the shared contribution of both genetic and environmental factors to diet-related polygenic disorders. Evidence for a genetic basis of the MetS and T2DM has been derived from studies of families, twins and populations with genetic admixture. The familial nature, the marked difference in the prevalence of the MetS among various racial groups and the difference in concordance rates between monozygotic twins are clearly consistent with a genetic component to disease susceptibility. High heritability estimates have been reported for fasting glucose, insulin, TAG and HDL cholesterol concentrations [24, 25]. Poulsen et al. [26] have studied the relative impact of genetic vs. environmental factors for the development of the components of the MetS amongst 303 elderly twin pairs. This study demonstrated that glucose intolerance, obesity and low HDL cholesterol concentrations are significantly higher among monozygotic twins than among dizygotic twins, indicating a genetic influence on the development on these phenotypes [26]. In contrast, the heritability estimates for hyperinsulinaemia, hypertension and hypertriglyceridaemia are low, indicating a more important environmental influence on these components of the MetS. More recently, results from a comprehensive study examining young and elderly monozygotic and dizygotic twins provided further evidence for a role of genes in controlling insulin secretion, insulin action and non-oxidative glucose metabolism [27]. These data indicate a major role for genetic susceptibility, whilst also emphasising the important role of the environment. In particular, trends in diet and physical activity, coupled with genetic susceptibility, must account for the recent and dramatic rise in the incidence of the MetS and T2DM, demonstrating the important impact of gene–environment interactions.

With the advent of high-throughput genetic analysis, our understanding of the genetic architecture and biology of diet-related polygenic disorders is improving. 2006 heralded identification of the most important T2DM susceptibility gene known so far, transcription factor 7-like 2 (TCF7L2) [28]. The original study in Icelandic, Danish and US white cohorts reported that single nucleotide polymorphisms (SNPs) of the TCF7L2 gene, especially rs12255372 and rs7903146, were strongly associated with T2DM [28]. This finding has been widely reproduced in several populations and also been shown to predict the conversion from IGT to overt diabetes [29]. More recently a Finnish study investigated the mechanisms of how these SNPs increase T2DM risk. They found that these variants altered TCF7L2 gene expression and are associated with decreased insulin secretion, but not insulin sensitivity, both in the general population as well as in T2DM offspring [30]. As part of this research the Finnish Diabetes Prevention Study investigated the association of these SNPs with incident diabetes. Five hundred and twenty-two overweight subjects with IGT were randomly assigned to either an intensive diet and lifestyle intervention group or a control group. Subjects in the intervention programme were given individually tailored dietary advice aimed to reduce weight and the intake of total and saturated fat and to increase the intake of dietary fibre. In addition, subjects were guided to increase their level of physical activity. The control group received general information on the benefits of weight reduction, physical activity and healthy diet. The mean duration of follow-up was 3.9 years, at which point DNA for genotyping was available from 507 subjects. Interestingly they found that variants were associated with incident diabetes in the control group, but not the intervention group. This indicates that environmental factors such as lifestyle intervention can reduce the risk conferred by genetic factors, even when risk genotypes are related to impaired insulin secretion [30].

There is no doubt that development of high-density arrays that permit the genotyping of hundreds of thousands of polymorphisms will rapidly advance our understanding of the genetic basis of T2DM, the MetS and obesity. A recent study [31] tested 392,935 SNPs in a French T2DM case-control cohort (n = 1,363). Markers with the most significant difference in genotype frequencies between T2DM cases and controls were fast-tracked for testing in a second cohort (n = 5,511). In addition to confirming the known association with the TCF7L2 gene, this study identified four loci containing variants that confer T2DM risk. These loci include a non-synonymous polymorphism in the zinc transporter SLC30A8, which is expressed exclusively in insulin-producing β-cells, and two linkage disequilibrium blocks that contain genes potentially involved in β-cell development or function (IDE-KIF11-HHEX and EXT2-ALX4). These associations may in part explain a substantial portion of disease risk and constitute proof of principle for the genome-wide approach to the elucidation of complex genetic traits [31]. Of the significantly associated SNPs in the French study, a number of these associations have been replicated in an Icelandic population in which 313,179 SNPs were tested for association with T2DM in a sample of 1,399.
T2DM patients and 5,275 controls [32]. This study found that the TCF7L2 SNP rs7903146 conferred the most significant risk. In addition to confirming the association between the risk variants of SLC30A8 and HHEX with T2DM they also identified a variant in CDKAL1 that was associated with T2DM in European ancestry and Han Chinese ancestry. The insulin response for homozygotes was about 20% lower than for heterozygotes or non-carriers, indicating that this variant confers risk of T2DM through reduced insulin secretion. While the function of the gene product of CDKAL1 is unknown, the protein product is similar to another protein, CDK5 regulatory subunit-associated protein 1 (encoded by CDK5RAP1). In pancreatic β-cells CDK5 has been shown to play a role in the loss of β-cell function under glucotoxic conditions [33]. It is tempting to speculate that CDKAL1 may facilitate insulin production under such conditions through interaction with CDK5.

Genome-wide studies for T2DM susceptibility genes have also been useful in uncovering genes associated with obesity, a key feature of the MetS. Recently a common variant in the FTO gene that predisposes to diabetes through an effect on body mass index (BMI) has been identified [34]. This study compared 1,924 UK T2DM patients with 2,938 UK population controls for 490,032 autosomal SNPs. Polymorphisms in the FTO gene region on chromosome 16 were strongly associated with T2DM. They replicated this association in a further 3,757 T2DM cases and 5,346 controls. The 16% of adults who are homozygous for the risk allele weighed about 3 kg more and had a 1.67-fold increased risk of obesity when compared to those not carrying a risk allele. Furthermore the authors studied the association of FTO gene variation with BMI and the risk of being overweight and obese in an additional 19,424 white European adults and 10,172 white European children. These studies revealed that the T2DM-associated A allele of rs9939609 was associated with increased BMI and increased risk of being overweight and also being obese from childhood into old age [34]. While the function of the FTO gene is unknown, it lies adjacent to another gene, KIAA1005, which is transcribed in the opposite direction, opening up the possibility that genetic variation may affect a regulatory element for KIAA1005. Understanding the mechanisms whereby these variants influence the risk of obesity may provide insights into novel pathways involved in adiposity.

Prior to high-throughput large scale whole-genome-wide studies, genome scanning by positional cloning and the candidate gene approach have been the traditional techniques for identification of genes associated with T2DM and the MetS. Positional cloning involves mapping the susceptibility or causative loci purely on their chromosomal location using multi-generational pedigrees and/or a large number of sibling pairs. This approach allows identification of genes without any previous knowledge of biological function or the disease mechanism. However, to date this approach has identified relatively few candidate genes relevant to the MetS. The hepatic nuclear factor 4α (HNF4α) gene partly explains the linkage peak on chromosome 20 [35], while the upstream transcription factor (USFI) is associated with familial combined hyperlipidaemia and maps close to the T2DM-associated 1q peak [36–38]. Genome scanning in several different ethnic groups has identified chromosome regions harbouring T2DM susceptibility genes such as the novel gene, calpain 10 (CAPN10) [39]. Subsequent to this initial report, several groups have failed to show a relationship between CAPN10 and metabolic traits, or have shown modest associations for other sequence variations and/or haplotype combinations at the CAPN10 locus that might be associated with disease [40]. The lack of a consistent gene–phenotype relationship may be related to a number of causes of inconsistency in association studies, including population-specific patterns of linkage disequilibrium, population-specific environmental triggers, gene–gene interactions or gene–nutrient interactions.

The candidate gene approach identifies genes according to biological function and/or linkage studies, and association tests for significant differences in their allele frequencies between a patient group and a control population. Given the number of metabolic pathways involved in the MetS it is immediately apparent that the number of potential candidate genes is tremendous and overall, there has not been a high extent of success from candidate gene studies in terms of defining the genetic determinants of the MetS. Table 1 summarises the key pathways involved and some of the relevant candidate genes.

Monogenic forms of diabetes have identified several candidate genes for T2DM and the MetS. Six genes, glucokinase (GCK), insulin promoter factor-1 (IPF-1), neuro D1 transcription factor (NEUROD1), HNF-1β and HNF-4α, account for the majority of the 34 monogenic forms of diabetes [41]. Candidate gene association studies in T2DM indicate a role for a number of genes involved in insulin action as well as β-cell function [42, 43]. Fasting hyperglycaemia in T2DM is associated with a lack of inhibition of two key gluconeogenic enzymes, phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-
Table 1. Pathways adversely affected in the metabolic syndrome, and some of their relevant genes

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Genes involved</th>
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<tr>
<td><strong>Insulin signalling</strong></td>
<td>Forkhead box protein 1α (FOXO1A), glucose transporter 4 (SLC2A4), glycogen synthase kinase-3 (GSK3), insulin (INS), insulin promoter factor (IPF1), insulin receptor (INSR), insulin receptor substrates 1 and 2 (IRS1 and IRS2), phosphoinositide-3-kinase, regulatory subunit 1 (p85α) (PIK3R1), phosphoinositide-3-kinase, regulatory subunit 2 (p85β) (PIK3R2), protein kinase Bβ (AKT2), potassium channel inwardly rectifying subfamily J member 11 (KCNJ11), protein tyrosine phosphatase 1B (PTPN11), phosphatase and tensin homolog (PTEN), SH2-containing inositol phosphatase 2 (INPP2B), Son of sevenless homolog 1 (SOS1)</td>
</tr>
<tr>
<td><strong>Glucose homeostasis</strong></td>
<td>Calpain 10 (CAPN10), forkhead box O3A (FOXO3A), glucagon receptor (GCCR), glucokinase (GCK), glucose-6-phosphatase (G6PC), glucose transporters 2 and 4 (SLC2A2 and SLC2A4), glucose 6-phosphate transporter (SLC2A7), glycogen synthases 1 and 2 (GYS1 and GYS2), glycogen synthase kinases 3α and 3β (GSK3A and GSK3B), hexokinase 2 (HK2), hepatic nuclear factor 1α (TCF1), hepatic nuclear factor 1β (TCF2), hepatic nuclear factor 4α (HNF4A), phosphoenolpyruvate carboxykinase 1 (PCCK1), upstream stimulatory factor 1 (USF1)</td>
</tr>
<tr>
<td><strong>Lipo-protein metabolism</strong></td>
<td>ATP-binding cassette, subfamily A, member 1 (ABCA1), ATP-binding cassette, subfamily G, member 5 (ABCG5), ATP-binding cassette, subfamily G, member 8 (ABCG8), adipoproteins (APOA1, APOA2, APOA4, APOA5, APOB1, APOC2, APOC3, APOC4, APOE), acetoacetyl-CoA thiolases 1 and 2 (ACAT1 and ACAT2), β1- and β2-adrenergic receptors (ADRB1 and ADRB2), CCAT-enhancer-binding protein α (CEBPA), intestinal fatty acid-binding protein (FABP2), farnesoid X-activated receptor (NR1H4), liver X receptor α (LXRA), adiponectin lipase (LPL), peroxisome proliferator-activated receptors α, δ, and γ (PPARA, PPARD and PPARG), PPARγ co-activators 1α and 1β (PPARGC1A and PPARGC1B), tissue type plasminogen activator (PLAT), endothelin receptor (EDN1), von Willebrand factor (VWF)</td>
</tr>
<tr>
<td><strong>Adipogenesis and inflammation</strong></td>
<td>Adipocyte fatty acid-binding protein (FABP4), adiponectin (ADIPOQ), CCAT-enhancer-binding proteins α and β (CEBPA and CEBPB), ghrelin (GHRH), interleukin 6 (IL6), leptin (LEP), receptor leptin (LEPR), resistin (RETN), tumour necrosis factor α (TNF), uncoupling proteins 1 and 3 (UCP1, UCP2 and UCP3)</td>
</tr>
<tr>
<td><strong>Vascular function and coagulation</strong></td>
<td>Angiotensinogen (AGT), angiotensin-converting enzyme (ACE), β1- and β2-adrenergic receptors (ADRB1 and ADRB2), endothelial nitric oxide synthase (NOS3), fibrinogen α and β polypeptides (FGA and FGB), indole-3-acetylindole (NOS2A), plasminogen activator inhibitor type 1 (SERPINE1), tissue plasminogen activator (PLAT), endothelin-1 (EDN1), von Willebrand factor (VWF)</td>
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phosphatase (G6Pase) which are regulated at the transcriptional level by insulin through protein kinase B (PKB) [44]. The essential role of PKB (or AKT2) in insulin signalling and maintenance of glucose homeostasis was demonstrated by the finding of a missense mutation in AKT2 in a family with severe insulin resistance and diabetes [45]. PKB also regulates the peroxisome proliferator-activated receptor α (PPARα) coactivator (PGC1α) [46], the transcriptional co-activator which interacts with HNF4α, PPARγ and forkhead transcription factor 1 (Foxo1) to execute hepatic gluconeogenesis. Impaired insulin signalling and glucose metabolism within the context of insulin resistance have been comprehensively reviewed elsewhere [47]. This review will focus on recent advances of well-characterised, common genetic variants of lipid-related genes associated with the MetS.

Dyslipidaemia is one of the very early features of the MetS and frequently precedes IGT. A number of lipid-sensitive transcription factors, including FXR, LXRα, RXRα, PPARα, PPARβ, PGC1α, PGC1β, SREBP-1a and SREBP-1c, have been implicated in the development of the MetS. PPARγ is a good candidate gene for the MetS because of its multiple roles in adipocyte differentiation, fatty acid metabolism, insulin sensitivity and glucose homeostasis [48–50]. Up until recently the Pro12Ala PPARγ polymorphism had been identified as the most widely reproduced genetic variation for the risk of T2DM [40]. The original study investigating the polymorphism in T2DM demonstrated that the alanine allele of the Pro12Ala PPARγ polymorphism was associated with lower BMI, improved insulin sensitivity and thus reduced diabetes risk by 75% [51]. However not all associations with this polymorphism were consistent [52–55] and those reports that have confirmed the association [56] have shown that the apparent protective effect of the PPARγ polymorphism is to a much lesser extent. Thus, a large association study of 3,000 subjects supported by a meta-analysis of 16 previously published studies has been conducted to overcome the inherent limitations of the smaller individual studies [57]. This study confirmed a modest (1.25-fold) but significant increase in diabetes risk for the Pro12Pro genotype [57]. More recently the contribution of the Pro12 allele to the genetic risk of T2DM was confirmed in a French Caucasian population, the Pro12 allele almost doubled risk particularly in obese subjects. Interestingly the obese phenotype seemed to exacerbate the detrimental effect of the PPARγ Pro12 allele on insulin sensitivity [58]. The Pro12Pro genotype also predicts the conversion from IGT to T2DM, Pro12 homozygotes had a 2.9-fold greater risk of developing diabetes compared to Ala12 carriers [59]. Haplotype studies have demonstrated that the Ala12 variant of PPARγ modulates susceptibility to T2DM in concert with other variants at the PPARγ locus. Doney et al. [60] showed that the Ala12 variant conferred...
protection in haplotypes not containing the 143IT variant, but the presence of 143IT in the haplotype (70% of Ala12 carriers) abolished protection. Interestingly, another recent study of four common PPARγ polymorphisms (−681C/T, −689C/T, Pro12Ala, and 1431C/T) found no impact of individual polymorphisms on the MetS. However haplotype analyses revealed a significant enrichment of the GTGC haplotype frequency (constituted by the −681C/T, −689C/T, Pro12Ala, and 1431C/T polymorphisms in this order) among subjects with the MetS [61]. These data support the suggestion that PPARγ gene variation may increase the risk of the MetS.

Disturbed lipoprotein metabolism is a key feature of the MetS, characterised by elevated TAG and low HDL cholesterol concentrations. One of the new potential candidate genes is the scavenger receptor class B type I (SR-BI or SCARB1) gene. SCARB1, a cell-surface glycoprotein, was the first HDL receptor to be defined and characterised. Acton et al. [62] described three common variants (at exon 1 [G/A], exon 8 [C/T], and intron 5 [C/T]), which were associated with HDL cholesterol, triglycerides, and BMI, suggesting that SCARB1 might be involved in determining some features of the MetS. In terms of the relations between HDL and triglyceride-rich lipoproteins (TRLs), the lipase gene family (hepatic lipase (LIPC), lipoprotein lipase (LPL), endothelial lipase (LIPG), and pancreatic lipase (PL)) represents a growing and promising superfamily in which common variations have been related to HDL cholesterol and TAG metabolism, but also sporadically with blood pressure, obesity, and insulin resistance. Numerous variants within the LPL gene, which hydrolyses core TAG from circulating TRLs that are then either degraded by the liver or converted to LDL particles by LIPG, have been identified (i.e., HindIII, S447X, D9N, and N291S), and they have been widely associated with HDL cholesterol and TAG concentrations [63]. However, some differences among studies and populations suggest the presence of interactions with additional factors [63]. A recent study examined the associations of LIPG haplotypes with lipoprotein risk factors in 541 adult Japanese Americans and found stronger associations for HDL₃ cholesterol and plasma apolipoprotein AI levels [64]. Numerous polymorphisms have also been analyzed in the hepatic lipase gene. Four SNPs in the promoter region (−250G/A, −514C/T, −710T/C, and −763A/G) are in strong linkage disequilibrium, and they have been associated with HDL cholesterol and TAG levels, with important differences among studies depending on the ethnic, anthropometric, and dietary characteristics of the populations [65].

Finally, several variants of the APOAI/C3/A4/A5 and APOE/C1/C2 gene clusters have been consistently associated with the characteristic dyslipidaemia of the MetS [66]. Lai et al. [67] have investigated five polymorphisms of the newly identified ApoAV gene, which lies close to the APOAI/CIII/AIV gene cluster, in several Asian populations and in the Framingham population, which is mostly of European descent. In all populations, the −1131T/C polymorphism had an important impact on plasma TAG and HDL cholesterol. In addition, the rare alleles of the −1464T/C, −1131T/C, S19W, and 1259T/C SNPs were each significantly associated with elevated TAG levels (19–86 mg/dl) and had clear gene-dose effects [68]. The Ala54Thr polymorphism in the fatty acid-binding protein 2 (FABP2) gene as well as the −455T/C and −482C/T polymorphisms in the apolipoprotein C-III (APOC3) gene promoter have been associated with features of the MetS in specific populations [69]. Guettier et al. [69] evaluated the association between these polymorphisms in Asian-Indians with the MetS and dyslipidaemia. Controls carrying FABP2 Thr54 were more likely to have the MetS than non-carriers. Those carrying at least one polymorphic allele in both genes had a higher likelihood of having the MetS and dyslipidaemia than wild-type [69].

In conclusion these data indicate a major role for genetic susceptibility to the MetS. However, regardless of the approach used, genetic disease-association studies are fraught with difficulties, and a number of the positive results have not been replicated in subsequent studies [70]. The main reasons for the disparity are inadequate statistical power, multiple hypothesis testing, population stratification, publication bias and phenotypic differences. It is becoming increasingly evident that the identification of true genetic associations in common multi-factorial conditions, such as the MetS, requires large studies consisting of thousands rather than hundreds of subjects. Also, the absence of large single-gene effects and the detection of multiple small effects accentuate the need for the study of larger populations in order to reliably identify the size of the effect now expected for complex diseases. The discovery of associated variants in unsuspected genes and outside coding regions illustrates the ability of genome-wide association studies to provide potentially important clues into the pathogenesis of common diseases. However it is also important to bear in mind that the human genome has not significantly changed in the last decade but the prevalence of the MetS has increased exponentially, illustrating the importance of gene–environment interactions.
Nutrient Determinants of the Metabolic Syndrome – Dietary Fat Composition

In addition to elevated fasting glucose concentrations, the insulin-resistant subject displays an imbalance in fatty acid metabolism reflected by increased levels of circulating NEFAs and TAG. This metabolic phenotype is exacerbated in the obese state [71] and inhibits insulin-stimulated glucose uptake into muscle. In this insulin-resistant state, NEFA levels are increased both in the fasted and the fed state [72]. In obesity, adipose tissue is resistant to the anti-lipolytic effect of insulin, and becomes limited in its ability to store lipids. Consequently, an increase in circulating NEFAs occurs leading to an imbalance between the oxidation of fat and of glucose [73]. It has been proposed that abnormal accumulation of fat in muscle and other tissues leads to lipotoxicity, induced β-cell failure, hyperglycaemia, increased plasma NEFA concentrations and increased VLDL production, all playing an important role in the aetiology of insulin resistance [74].

NEFAs have an important stimulatory effect on glucose-stimulated insulin secretion (GSIS) by maintaining a basal rate of insulin secretion and in turn regulating adipose tissue lipolysis. In the MetS and T2DM, increased NEFAs contribute to hyperinsulinaemia and ultimately overexposure results in β-cell damage. It has been proposed that elevation in the basal glucose level increases glucose-derived malonyl-CoA within the β-cell [75]. Increased levels of intracellular malonyl-CoA inhibit the activity of carnitine palmitoyl transferase-1 (CPT-1) resulting in increased long-chain acyl-CoA levels [76]. The malonyl-CoA–CPT-1 interaction is central to GSIS, highlighting the role of fatty acids in the process [74]. The insulinotrophic effects of NEFAs differ with respect to the level of saturation. In one study, endogenous circulating NEFAs were eliminated with the anti-lipolytic agent nicotinic acid and replaced with soybean oil (which is predominantly polyunsaturated fatty acids (PUFAs)) or lard oil (a source of saturated fatty acids (SFAs)). After a glucose challenge, the proportion of insulin secretion was far more effective after the SFA treatment [77]. This study also demonstrated that GSIS increased dramatically with chain length and degree of unsaturation (C8:0, C18:2, C18:1, C16:0, C18:0). Other animal studies have displayed a potent effect of increased islet TAG content on β-cell function [78]. This fat accumulation in the islets and its subsequent effects on GSIS warrants further investigation.

Disruption of the insulin-signalling cascade by increased NEFA- or fatty acid-derived products including TAG has a negative impact on insulin sensitivity. These effects are well characterised in muscle [79]. The insulin-signalling cascade involves a sequence of reactions in which binding of insulin to the insulin receptor (IR) causes tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1). Subsequent activation of phosphatidylinositol (PI) 3-kinase facilitates translocation of glucose transporter 4 (GLUT4) to the plasma membrane resulting in increased glucose transport into the cells [80]. Circulating NEFAs (malonyl-CoA, long-chain acyl-CoA) reduce muscle glucose oxidation and glycogen synthesis by impairing glucose transport [81] and impeding insulin-mediated tyrosine phosphorylation of IRS-1 and IRS-1-associated PI3 kinase activity, which is associated with a substantial increase in membrane-bound protein kinase C theta (PKCθ), a known serine kinase. LC-acyl-CoA generates a pool of diacylglycerol which may activate PKCθ. Phosphorylation of IRS-1 on a serine residue affects GLUT4 translocation to the cell surface [74]. A similar mechanism involving IRS-2 is suggested to occur in the liver [80]. As the IR is a membrane-bound protein and its function is membrane-dependent, it is reasonable to speculate that a change in membrane lipid induced by dietary fat may impact on the function of the plasma membrane IR [81]. In isolated rat adipocytes, it has been demonstrated that a high PUFA:SFA diet increased IR function, glucose oxidation and glucose transport [82]. A high-PUFA diet can also increase receptor tyrosine kinase activity [83]. Again these dietary effects may be further modulated by the degree of unsaturation and chain length of the unsaturated fatty acids.

There is increasing evidence that the composition of the diet, in terms of quality and quantity of fat, plays an important role in glucose homeostasis and insulin sensitivity. It is generally agreed that saturated fats have a detrimental effect on lipoproteins and on insulin sensitivity whilst unsaturated fats have a more beneficial outcome. Animal and human studies have demonstrated that SFAs increase insulin resistance [84–87]. Studies determining the effect of monounsaturated fatty acids (MUFAs) on insulin resistance have demonstrated improved peripheral insulin sensitivity following MUFAs-rich diets in both healthy [88, 89] and diabetic cohorts [90]. However, the effect of MUFAs on insulin resistance is still somewhat controversial since it has been suggested that dietary oleic acid influences fat oxidation [91], which may in turn have a negative effect on insulin sensitivity. The
role of n–6 and n–3 PUFAs on insulin sensitivity remains controversial [6, 92].

While there is clear evidence from epidemiological and cohort studies of the detrimental effects of total fat (particularly SFA) on insulin resistance and the development of T2DM [93–96], the optimal amount and type of dietary fat for the prevention of insulin resistance and the MetS remains unclear. Diets rich in MUFAs tend to improve insulin sensitivity [97, 98], however not all studies have shown a positive effect [91, 99]. A number of studies have also focused on the relative effect of substituting SFA with MUFA or carbohydrate, since high-carbohydrate diets have been associated with increased TAG concentrations, reduced HDL cholesterol concentrations [90, 100] and reduced glycaemic control.

The anti-atherogenic effects of PUFAs are well documented [101]. However their effects on insulin sensitivity are less well defined. In vitro and animal studies have shown encouraging effects of PUFAs on insulin action [102], especially with fish oils of which long-chain n–3 PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are found in high concentrations [103]. Laaksonen et al. [104] showed an association of the n–6 PUFA linoleate in serum with the risk of development of impaired fasting glucose or diabetes in middle-aged men over a 4-year follow-up. Salmeron et al. [105] postulated a reduction in T2DM by replacing 2% of energy from trans fatty acids isoenergetically with PUFAs, indicating that increased intakes of long-chain PUFAs reduce insulin resistance. Animal studies have confirmed this, showing significant decreases in lipids, glucose levels and an increase in insulin sensitivity [106]. Intake of fish oils directly influences the fatty acid composition of membrane phospholipids [103] and increases insulin sensitivity [107, 108]. n–3 PUFAs require sufficient duration to effect a compositional change in cell membrane phospholipids in order for their direct action on insulin resistance to be assessed, in contrast to their established indirect effect on endothelial function, platelet aggregation, TAG concentration and arrhythmic effects [109]. Animal studies have shown that a diet rich in long-chain n–3 PUFAs (EPA and DHA) prevented insulin resistance in muscle and liver, through the hypothesised effect of reducing fat content in muscle and thus maintaining normal PI3 kinase activity and expression and translocation of GLUT4 receptors and by inhibiting hepatic glucose production in the liver [84].

In contrast there is limited and conflicting epidemiological data regarding the effects of fish and fish oil supplements in humans on insulin sensitivity. The Seven Countries Study [110] and the Nurses Health Study [105] showed inverse associations between fish consumption and glucose concentrations and the incidence of T2DM. However other studies have failed to replicate these findings [111]. In the KANWU study, supplementation with n–3 PUFAs did not influence insulin sensitivity on the high-fat or high-MUFA diets, confirming the controversial effects of n–3 PUFAs in human interventions as opposed to animal models.

Numerous intervention studies determining the effect of total fat intake on insulin sensitivity (measured by the clamp technique or variations in the frequently sampled intravenous glucose tolerance test) have shown that high-fat diets (>38% energy from fat) exacerbate insulin resistance. Low-fat diets, however, have been shown to have a beneficial effect [99, 112–114]. Categorising people by BMI was shown to increase insulin sensitivity in the more overweight and obese categories when total dietary fat is reduced [91]. The use of 24-hour recalls and weighed food diaries in estimating diet composition are adequate but not ideal. Underreporting of dietary fat, carbohydrate, and frequency of snacking and over-emphasis of a healthy eating type pattern is repeatedly observed in dietary studies, especially in overweight and obese recorders. As a complementary and more reliable or valid marker of habitual diet, fatty acid composition of serum lipid esters can be measured. Vessby [115] found higher proportions of SFAs in T2DM patients compared with healthy controls. Vessby et al. [116] subsequently measured the fatty acid composition of serum cholesterol esters, representative of the dietary fat composition for the previous 6–8 weeks, in 70-year-old men. Insulin sensitivity was associated with low proportions of palmitic (C16:0) and palmitoleic (C16:1n–7) acids and a high proportion of linoleic acid (C18:2n–6) [116]. The fatty acid profiles of the insulin-resistant subjects suggest possible changes in the activities of desaturation (Δ5-desaturase, Δ6- and Δ9-desaturase) and elongation enzymes [115] indicating that these enzymes are insulin-dependent. These desaturase activity profiles have also been observed with obesity and lifestyle factors in men and women [117]. Clearly disturbed fatty acid metabolism, which may be secondary to excessive/imbalanced dietary fat intake, may be involved in the pathogenesis of the MetS. More recently, Warenso et al. [118] identified specific fatty acid factors as measures of dietary fat quality and endogenous fatty acid metabolism in relation to the MetS. Using principal factor analysis on fatty acid profiles of men participating in a population-based cohort study, the Uppsala Longitudinal Study of Adult Men, factors were generated at ages 50 (n = 2,009) and 70 (n = 576) years, and relations between
fatty acid factors and the MetS (National Cholesterol Education Program) were studied in cross-sectional and prospective (20 years) analyses. They identified 3 major fatty acid factors: a low-linoleic acid factor, a dietary saturated fatty acid factor, and an n–3 polyunsaturated fatty acid factor. All factors differed between those subjects with the MetS (n = 281 of 2,009) and those without the MetS at age 50 years; only the low-linoleic acid factor differed at age 70 years, suggesting an association between the MetS and fat quality. The low-linoleic acid factor and the n–3 PUFA factor predicted the development of the MetS over 20 years, independent of smoking habits, physical activity, and BMI. This finding supports current dietary recommendations to increase PUFA and restrict SFA intakes.

**Gene–Nutrient Interactions: Implications for Dietary Fatty Acids**

In light of the Human Genome Project and the rapid advances in molecular biology, a wealth of genetic information is being generated, particularly with respect to the common, polygenic, diet-related diseases including obesity, insulin resistance, the MetS and T2DM. It is becoming increasingly obvious that an individual’s phenotype represents a complex interaction between the genetic background and environmental factors over the course of an individual’s lifetime. Food intake and nutrient exposure are key environmental factors in the pathogenesis and progression of the common polygenic, diet-related diseases. Therefore it is time to identify the nutrient-sensitive genotypes and to develop a ‘personalised nutrition’ approach, whereby nutrient intake is manipulated/optimised based on an individual’s genetic profile to reduce disease risk and/or improve the effectiveness of dietary guidelines/recommendations.

It is well known that the effect of dietary changes on plasma biomarker concentrations differs significantly between individuals. Currently, there is considerable support for the notion that the inter-individual variability in response to dietary modification is determined by genetic factors – this is especially true for lipid and lipoprotein phenotypes [119]. Insulin resistance precedes the development of MetS and T2DM. Genes and the environment determine insulin resistance. The ‘thrifty genotype’ [120], arising from evolutionary selection of genes that were originally beneficial for energy storage and which conferred a protective effect in times of food deprivation by promoting fat deposition, has been proposed to explain the current escalating incidence of obesity in a modern environment of physical inactivity and excessive energy intake. This review will explore the interactions between genetic background and an individual’s nutrient exposure, with particular focus on dietary fatty acid composition.

As previously mentioned hepatic lipase (HL) is a lipolytic enzyme that plays a role in the metabolism of several lipoproteins, while insulin up-regulates the activity of HL via insulin-responsive elements in the HL promoter. Results from the Hoorn Study suggest that higher intakes of total and saturated fat were positively associated with higher HL activity. Furthermore they reported that the observed association between total fat and HL activity was modified by the −514C/T polymorphism of the hepatic lipase gene, with stronger associations observed between total dietary fat intake and HL activity in subjects with the CT and TT genotypes compared to CC homozygotes [121]. Data from NUGENOB, a study investigating genotype-by-nutrient interactions in obesity, in which 42 polymorphisms of 26 candidate genes for obesity were genotyped in 549 adult obese women recruited from eight European centres in a case-only study, reported an interaction between the −514C/T HL gene polymorphism and fibre intake. They also found interactions between the −11377G/C polymorphism of the adiponectin gene (ADIPOQ) and the −681C/G polymorphism of the PPARG3 gene, with the percentage of energy derived from fat in the diet for the development of obesity. However it must be noted that this study was case-only in design and did not take multiple testing into account, therefore these results should be considered with caution [122]. Orlov et al. [123] investigated the interaction effects between the −514C/T polymorphism, dietary fat and HDL-related measures in 1,020 men and 1,110 women participating in the Framingham Study. They found that the T allele was only associated with higher HDL cholesterol levels and size in subjects consuming <30% of energy from fat. When total fat was ≥30% of energy mean HDL cholesterol levels were lowest in the TT homozygotes and no difference was observed in the CT and CC individuals. These correlations were observed for SFA and MUFA (particularly for animal fat), but not for PUFA. One interpretation of these results could be that in the Framingham Study, TT subjects may have an impaired adaptation to higher animal fat diets and could result in higher cardiovascular risk.

Gene–diet interactions between the APOA5 gene variation and PUFAs in relation to plasma lipid concentrations and lipoprotein particle size have been reported [124]. Furthermore, it has been shown that obesity modu-
lates the effect of the APOA5 gene variation in carotid intima media thickness, a surrogate measure of atherosclerosis [125]. More recently the same group reported that the −1131T/C polymorphism in the APOA5 gene modulates the effect of fat intake on BMI and obesity risk in both men and women in the Framingham Study [126]. The interaction with BMI was dose-dependent, and no statistically significant heterogeneity by gender was detected. In subjects homozygous for the −1131T major allele, BMI increased as total fat intake increased. Conversely, this increase was not present in carriers of the −1131C minor allele. When specific fatty acid groups were analyzed, MUFAs showed the highest statistical significance for these interactions with obesity-related measures (BMI, overweight, and obesity).

Apolipoprotein CIII (apo CIII) is a marker of CVD risk associated with TAG-rich lipoproteins. The −455T/C polymorphism in the insulin-responsive element of the apo CIII gene influences TAG and apo CIII concentrations. A formal interactive effect between apo CIII genotype and n–3 PUFA was confirmed by logistic models in the study of Oliveri et al. [127]. Patients homozygous for the −455C apo CIII variant are poorly responsive to the apo C-III-lowering effects of n–3 PUFA [127].

Apolipoprotein E is a structural component of several lipoproteins, and serves as a ligand for the LDL receptor and the LDL receptor-related protein. The APOE gene promoter −219G/T polymorphism is associated with coronary heart disease and increased postprandial TRL levels [128], and susceptibility of LDL to oxidative modifications [129], circumstances related to insulin resistance. The effect of this polymorphism on peripheral insulin sensitivity was investigated using the insulin suppression test after the consumption of three diets: high MUFA, high SFA and high carbohydrate. The steady-state plasma glucose concentration was lower in GG subjects than in GT and TT individuals, regardless of the diet consumed. Significant diet–genotype interactions were found for steady-state plasma glucose and NEFA concentrations. Thus the shift from the SFA-rich diet to the MUFA- or carbohydrate-rich diets decreased the steady-state plasma glucose and NEFA concentrations in GG and GT subjects, but not in TT subjects [130].

Scavenger receptor class B type I (SCARB1) mediates the absorption of dietary cholesterol in the intestine, suggesting that it may also play a role in postprandial responses. The presence of the 2 allele at the SR-BI polymorphism in exon 1 was associated with faster clearance of small TRLs, probably due to a more rapid hepatic uptake [131]. Increasing evidence indicates that SCARB1 plays additional roles particularly in T2DM. More recently, it has been reported that carriers of the G/A genotype have significant increases in insulin sensitivity after a MUFA-rich diet compared with G/G individuals [132].

The intestinal fatty acid-binding protein (IFBP), coded by the FABP2 gene, is one of the most abundant proteins in enterocytes and genetic variation at this locus was associated with insulin resistance in Pima Indians. In particular the Ala54Thr polymorphism has been associated with hypertriglyceridaemia and insulin resistance. Marin et al. [133] showed that insulin sensitivity decreased in subjects with the Thr54 allele when SFAs were replaced by MUFAs and carbohydrates. However, no significant differences between the 3 diets were observed in the Ala54 allele homozygotes.

PPARs are members of the nuclear-hormone receptor superfamily of which there are three isoforms PPARα, PPARβ/δ and PPAR-γ. Unsaturated fatty acids can bind to specific isoforms and activate PPARs. This binding varies according to the PPAR's affinity which is dependent on fatty acid chain length and degree of unsaturation. Recent studies have suggested that n–3 fatty acids protect against high-fat diet-induced insulin resistance through PPAR activation and a subsequent decrease in intracellular lipid abundance [134]. Tai et al. [135] have shown that the Leu162Val polymorphism of the PPARα gene effects plasma TG and apoC-III concentrations depending on the dietary PUFA, with a high intake triggering lower TG in carriers of the 162V allele. Furthermore, the PPARα Leu162Val polymorphism may contribute to inter-individual variability in plasma lipoprotein and lipid response after modification of the dietary PUFA:SFA ratio [136].

It has been demonstrated that several n–3 and n–6 PUFA activate PPARγ [137]. In the context of insulin resistance, PPARγ regulates adipogenesis and is involved in insulin sensitisation [137]. PPARγ promotes the storage of fat and increases adipocyte differentiation and enhances the transcription of genes important for lipogenesis [138]. The PPARγ Pro12Ala polymorphism provides an excellent example of the relevance of gene–nutrient interactions in the development of the MetS and T2DM. In a prospective population-based cohort study, Luan et al. [139] demonstrated an important interaction between habitual dietary fat composition and the PPARγ Pro12Ala polymorphism. They found that as the PUFA:SFA ratio increased a significant inverse relationship was shown for both fasting insulin concentrations and BMI.
in the Ala carriers, suggesting that the potential protective effect of the Ala allele may be lost in the presence of a high SFA diet. The Quebec Family Study [140] has also demonstrated that the PPARγ Pro12Ala polymorphism modulates the relationship between dietary fat intake and components of the MetS. In this study it was found that total fatty acid and SFA intakes are correlated with BMI, visceral adipose tissue area, waist circumference and fasting glucose concentrations in Pro12 heterozygotes, but these associations are not observed among carriers of the Ala allele. Also, when the two genotype groups are classified according to quartiles of total fatty acid and SFA intakes, the positive correlation between fat and waist circumference remains in the Pro12 homozygotes, but again there is no relationship in the Ala carriers [140]. Thus, there is a disparity between the results of these two studies that have investigated the interaction between the PPARγ Pro12Ala polymorphism and dietary fatty acids. While the discrepancies may be population-specific, both studies highlight the need to take account of diet-related environmental exposure and the relevance of gene–nutrient interactions, particularly in relation to the possible role of dietary fatty acids in the MetS. More recently, a direct relationship between the intake of trans fatty acids and T2DM in the Ala carriers of the polymorphism has been reported [141]. The authors speculate that from a biological point of view it is plausible for trans fatty acids to have the same effect as saturated fatty acids in activation of the steatosis pathways that result in insulin resistance and early β-cell dysfunction.

It is worth noting that while other studies have also reported this gene–nutrient interaction [140] the results have not been consistent with the original findings of Luan et al. [139], and others have failed to show a gene–nutrient interaction between the PPARγ polymorphism and dietary fatty acid composition [142]. One study also reported that the Ala12 allele was associated with an increased risk of progression to T2DM, however the Ala12Ala genotype was associated with increased weight loss in response to lifestyle intervention and less progression from IGT to T2DM [143]. More recently Scacchi et al. [144] analysed the distribution of this polymorphism and prevalence of T2DM in world populations in relation to dietary habits. In European populations the Ala allele frequencies are distributed according to a latitudinal trend, with the highest in northern and central European populations and the lowest in the Mediterranean populations. An inverse relationship between Ala frequency and T2DM prevalence was observed mainly in populations where energy from lipids exceeded 30% of the total energy intake. While the focus of this review is on environmental factors, in particular dietary fat, it should be noted that non-environmental factors should also be considered and recent studies have shown that physical activity interacts with the PPARγ Pro12Ala polymorphism and has effects on fasting insulin concentrations, a surrogate marker for insulin resistance [145, 146].

Conclusions

The recent global epidemic of the MetS is an important illustration of how nutrient exposure and genetic background can dramatically impact on the development of the disease. Clearly, there is ample evidence to suggest that an individual’s genetic background can interact with their dietary fat exposure to affect risk of the MetS. To date, most studies have focused on a limited number of genetic variants. More comprehensive genetic/nutrient analysis in larger cohorts is needed to confirm these findings. Nevertheless, the preliminary data provide an interesting concept worthy of further study, in terms of both gene–nutrient interactions modulating the risk of onset of the MetS and therapeutic dietary interventions. Indeed, in the future a ‘personalised nutrition’ approach may be advocated, wherein patients with a particular genetic profile(s) may determine responsiveness to specific dietary fatty acid interventions. With the advent of high-throughput genetic analysis our understanding of the genetic basis of diet-related polygenic disorders should improve, allowing the implementation of more effective patient risk assessment and personalised nutritional therapies. Only with a full understanding of multiple gene–gene, gene–nutrient and gene–nutrient–environment interactions can the molecular basis of the MetS be solved in order to reduce the risk and minimise the adverse health effects of obesity, T2DM and CVD.

Disclosure Statement

The authors declare that no financial or other conflict of interest exists in relation to the content of the article.
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Gene–Nutrient Interactions in the Metabolic Syndrome


99 Gene–Nutrient Interactions in the Metabolic Syndrome

J Nutrigenet Nutrigenomics 2008;1:136–151


122 Phillips/Tierney/Roche


Gene–Nutrient Interactions in the Metabolic Syndrome