Review

The potential role of olive oil-derived MUFA in insulin sensitivity

Audrey C. Tierney and Helen M. Roche

Nutrigenomics Research Group, School of Medicine, Institute of Molecular Medicine, Trinity College Dublin, St. James's Hospital, Dublin, Ireland

Dietary fatty acids play an important role in the development of insulin resistance, the prelude to type 2 diabetes mellitus. This review addresses the potential role of olive oil-derived MUFA in insulin sensitivity, particularly how dietary fat interacts with insulin resistance looking at whole body metabolic measures, as well as molecular effects. The review focuses on the role of non-esterified fatty acids, fatty acid composition *in vivo* and dietary fat modification on insulin resistance in the metabolic syndrome. Particular emphasis is placed on the role of olive oil within the context of dietary modification to improve insulin sensitivity and for the prevention of the metabolic syndrome.

Keywords: Fatty acids / Insulin sensitivity / Metabolic syndrome / Monounsaturated fat / Olive oil

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1 Introduction

1.1 Definition of insulin resistance and its importance in human health

In 1970, Berson and Yallow [1] 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". It results in an inability of insulin to provide normal glucose and lipid homeostasis. Reaven [2] hypothesised that insulin resistance, glucose intolerance and hyperinsulinaemia are the underlying components of the metabolic syndrome or "Syndrome X", and recently suggested that the more insulin-resistant a person, the more likely he or she will develop some degree of glucose intolerance, high triacylglycerol (TAG) and low HDL cholesterol concentrations, essential hypertension, and pro-coagulant and pro-inflammatory states, all of which increase the risk of cardiovascular disease (CVD) [3]. The vast majority of subjects with multiple metabolic disorders are insulin resistant, and in the general population insulin resistance exists in the absence of these metabolic disorders [4]. Zethelius *et al.* [5] proposed that insulin resistance is an independent risk factor for CVD, and, as the prevalence of obesity, type 2 diabetes mellitus (T2DM) and CVD are increasing to epidemic proportions [6], and significantly influence duration and quality of life [7], it seems paramount that, as a potential cause or preceding factor, insulin resistance should be studied in great depth to reach a full understanding of the underlying mechanisms and its interaction effects.

BMI and waist circumference correlate positively with insulin resistance [8, 9]. Waist circumference has been singled out as the most important risk factor in the new International Diabetes Federation (IDF) definition of the metabolic syndrome (available from www.idf.org). Importantly, the IDF criteria for the metabolic syndrome also accounts for ethnic-specific cut-offs for waist circumference. Therefore, the general consensus is that central adiposity is the most important determinant of insulin resistance, which further heightens the risk for T2DM and CVD. While diet and/or poor nutritional status is not a risk factor for the metabolic syndrome, 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. It has been demonstrated that weight loss in diabetic patients improves

Correspondence: Dr. Helen M. Roche, Nutrigenomics Research Group, School of Medicine, Institute of Molecular Medicine, Trinity College Dublin, St. James's Hospital, Dublin 8, Ireland
E-mail: hmroche@tcd.ie
Fax: +353-1-4542043

Abbreviations: **ATP**, Adult Treatment Panel; **CVD**, cardiovascular disease; **EPA**, eicosapentaenoic acid; **FFA**, free fatty acids; **GIP**, glucose independent insulinotrophic peptide; **GLP-1**, glucagon-like peptide-1; **GSIS**, glucose-stimulated insulin secretion; **HOMA**, Homeostasis Model Assessment; **IDF**, International Diabetes Federation; **IFG,** impaired fasting glycaemia; **IR**, insulin receptor; **IRS**, insulin receptor substrate; **IVGTT**, intravenous glucose tolerance test; **NCEP**, -National Cholesterol Education Program; **NEFA**, non-esterified fatty acids; **PI3K**, phosphatidylinositol 3-kinase; **PPAR**, peroxisome proliferator-activated receptor; **SFA**, saturated fatty acids; **TAG**, triacylglycerol; **T2DM**, type 2 diabetes mellitus

HbA1c percentages, which reflects fasting glucose levels during the previous 4 – 6 weeks, and improves insulin sensitivity in peripheral tissues by increasing the capacity of non-oxidative glucose metabolism [10]. It is probably that insulin resistance has both polygenic and environmental aetiology [11]. Since diet is one of the primary environmental factors, it makes sense to target diet in primary prevention programmes and in secondary treatments.

1.2 Measuring insulin sensitivity

As an underlying feature of the metabolic syndrome, it is critical to accurately assess insulin sensitivity *in vivo*. The euglycaemic hyperinsulinaemic clamp is deemed the "gold standard" for measuring insulin sensitivity [12]. Insulin is infused at a constant rate and glucose is maintained at a level between 5.0 and 5.5 mmol/L by glucose infusion. The rate of glucose infusion is a measure of insulin-mediated glucose disposal [13]. Another method, the Homeostasis Model Assessment (HOMA) is a mathematical model, which determines insulin resistance and β-cell function obtained from fasting insulin/C-peptide and fasting plasma glucose concentrations [14]. The advantage of HOMA is that it is a fast and effective way of assessing insulin resistance as sampling is simple and the outcome involves no complex computer modelling [15]. Another method to measure insulin sensitivity is the frequently sampled intravenous glucose tolerance test (IVGTT). This involves the injection of glucose at baseline and the measurement of plasma insulin and glucose at time points over a 3-h period. A modified protocol requiring the infusion of tolbutamide [16] or insulin 20 minutes after the glucose infusion now seems to be the preferred method as it allows an adequate endogenous plasma insulin response in the case of tolbutamide infusion and to augment a second-phase insulin secretion in individuals with impaired β-cell response in the case of the insulin infusion [12]. Mathematical modelling (MINMOD) is performed to provide an estimate of insulin sensitivity and other parameters [17, 18]. Other methods used to assess insulin sensitivity and insulin resistance are the insulin suppression test, the insulin tolerance test, and from indices derived from the data collected during an oral glucose tolerance test; fasting insulin, and quantitative insulin sensitivity check index [14, 15, 19 – 22] (Table 1).

1.3 Definitions of the metabolic syndrome – Importance of insulin resistance

While the clustering of the components that define the different versions of the metabolic syndrome is similar, each place a slightly different emphasis on the metabolic phenotype. The World Health Organisation (WHO) criteria focuses on risk for diabetes centred around impaired glucose tolerance, impaired fasting glucose or insulin resistance as measured by the hyperinsulinaemic euglycaemic clamp [23]. The IDF definition focuses on central adiposity, whereas the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults [Adult Treatment Panel (ATP) III] [24] assigns no priority to any of the criteria. In a recent perspective review, Reaven [3] summarised the similarities and differences of the three definitions of the metabolic syndrome (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 [25, 26], and from human metabolic studies including the insulin resistance atherosclerosis study [27 – 29].

1.4 Prevalence and economics

All defintions vary in their estimates of the prevalence of the metabolic syndrome, although the WHO and the ATPIII of the NCEP were shown to correlate closely in identifying people at risk of CVD [30]. The IDF definition tends towards higher prevalence rates of the metabolic syndrome [31, 32]. Recently, Sandhofer *et al.* [33] compared the three definitions with respect to metabolic parameters and their ability to predict intima media thickness and plaque extent in the carotid arteries. This group found an increased prevalence of the metabolic syndrome using the IDF criteria (25.8% for men and 19.5% for women). They 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 (HOMA) 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 subjects. In justifying their low cut-offs for waist circumference, the IDF Epidemiology Task Force Consensus Group made comparisons to the cut-offs used by the European Group for the Study of Insulin Resistance (EGIR). However, the EGIR requires insulin resistance as determined by hyperinsulinaemia [34]. As there is no universal definition of the metabolic syndrome, this hinders the determination of the true global prevalence both locally and internationally. As seen, the definitions vary considerably and not all include insulin resistance. Therefore, estimates of the prevalence of the metabolic syndrome will depend on the definition used. Using the NCEP definition, the prevalence of the metabolic syndrome is estimated to be 25% of the general population with no gender differences but varying with genetic background [35]. King *et al.* [36] estimated the prevalence of diabetes in adults to increase by 35% and the number of people with diabetes will increase by 122% by the year 2025. However, most of the studies used in the extrapolation of data were conducted in the 1980s, suggesting that

Table 1. Measures of insulin sensitivity

Technique	Sampling	Method	Outcomes
A			
Euglycaemic hyperinsulinaemic clamp [12, 15]	Blood glucose measurements every 3 – 5 min, in addition to plasma samples over 150 – 180 min	Constant rate IV insulin infusion calculated as a dose per unit of surface area and variable rate IV glucose infusion	Insulin resistance estimated from the ratio of the mean glucose infusion to the mean insulin concentration over the last 20 – 30 min
IVGTT with Minimal modelling [12, 15, 19]	12 – 35 samples taken over 180 min	IV glucose bolus, IV insulin/tolbutamide at 20 min	Analysis of results with MINMOD computer programme: estimates key indices of glucose-insulin dynamics: SI, insulin sensitivity; Sg, glucose effectiveness; AIRg, acute insulin response to glucose
Insulin suppression test [15]	six samples at 0, 60, 120, 150, 160, 170, 180 min	Constant rate infusion of somatostatin, glucose and insulin for 150 – 180 min	SSPG calculated from the mean of the glucose concentrations over the last 30 min
Insulin tolerance test [12, 15]	9 samples over 15 min	IV bolus of insulin	Logarithm of glucose concentrations plotted against time. IS estimated from the slope of the regression line
B			
Fasting Insulin [12, 20]	Average of 2 fasting samples		Logarithm usually best transformation
HOMA-IR [12, 14, 21]	3 basal samples at 5-min intervals		HOMA-IR= (fasting glucose (mmol/l) × fasting insulin (μU/ml))/22.5
QUICKI [22]	1 sampling occasion		QUICKI= [1/(log fasting insulin + log fasting glucose)]

A: Intravenous techniques.
B: indices derived from data collected during OGTT data; IV, intravenous; IVGTT, intravenous glucose tolerance test; IS, insulin sensitivity; HOMA-IR, Homeostasis model assessment-insulin resistance; SSPG, steady state plasma glucose; QUICKI, quantitative insulin sensitivity check index.

the results may be somewhat worse. Sunehag *et al.* [37] demonstrated that healthy obese adolescents were highly insulin resistant both in the fed and fasted state and after response to a glucose challenge. To maintain normal glucose and lipid metabolism obese adolescents required a twofold increase in their insulin secretion. This demand on the β-cell may lead to development of T2DM. This highlights the need to include such age groups in estimating prevalence of insulin resistant states.

As an integral component of the metabolic syndrome and T2DM, obesity and CVD, insulin resistance needs to be considered with the increasing prevalence of these largely preventable conditions. Healthcare costs associated with treatment and its implications are proving more and more of a burden on our economies. As reported by King *et al.* [36] the predicted number of middle-aged people (45 – 64 years) with diabetes was greater than elderly people (≥65 years), indicating long-term health care needs and costs.

The cardio-protective properties of the Mediterranean diet has received much attention in recent years and as the main source of dietary fat, monounsaturated fat derived mainly from olive oil is sure to have a major relevance in the different rates of CVD between countries and in particular of a north – south divide. The renowned Seven Countries Study [38] developed from the observation of the low rates of coronary heart disease in Southern Europe. A randomised trial in Lyon, France [39], in which the experimental diet was designed nutritionally similar to the traditional Cretan diet, high in α-linolenic acid and olive oil and the control diet was a low-fat diet suggested by the American Heart Association, showed a 70% reduction in the risk of recurrent CVD in the Mediterranean diet compared to the control group. The European Society of Cardiology 1999/2000 heart survey, EUROASPIRE II assessed patients in 15 countries (Belgium, Czech Republic, Finland, France, Germany, Greece, Hungary, Ireland, Italy, the Netherlands, Poland, Slovenia, Spain, Sweden and United Kingdom) and

delved into the marked differences in the rates of CVD across Europe and revealed rates higher in Eastern and Central European countries and lowest in Mediterranean countries [40] where olive oil is considered a staple. Diet and nutrition are sure to play a role in the realm of public health strategies to attenuate the increasing prevalence of both the metabolic syndrome, T2DM and CVD. As monounsaturated fat has been demonstrated in some studies to attenuate insulin resistance, as discussed below, advising a diet with monounsaturated fat-rich foods such as olive oil may be a means of reducing the incidence of the metabolic syndrome and its associated risk factors.

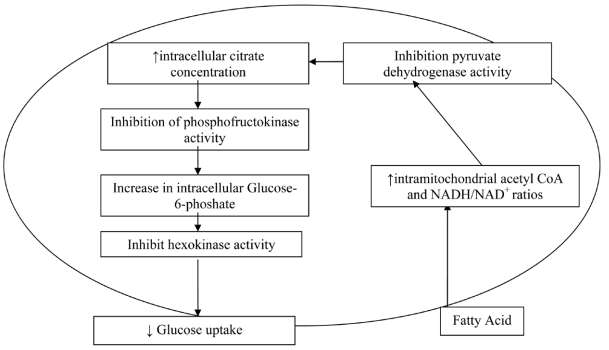

Figure 1. Fatty acid-induced insulin resistance in skeletal muscle, as proposed by Randle *et al.* [54].

2 Diet related determinants of insulin sensitivity

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, while unsaturated have a more beneficial outcome.

Animal and human studies have demonstrated that saturated fatty acids (SFA) increase insulin resistance [41 – 44]. The role of n-6 and n-3 PUFA on insulin sensitivity remains controversial [45, 46]. Studies determining the effect of MUFA on insulin resistance have demonstrated improved peripheral insulin sensitivity following MUFA-rich diets in both healthy [47, 48] and diabetic cohorts [49]. However, the effect of MUFA on insulin resistance is still somewhat controversial since it has also been suggested that dietary oleic acid influences fat oxidation [50], which may in turn have a negative effect on insulin sensitivity.

2.1 Effects of non-esterified fatty acids on insulin resistance

As the common feature of T2DM, insulin resistance also exists in the pre-diabetic state, therefore the molecular and metabolic aetiology of insulin resistance are the topic of many studies in the literature. Although previously a glucose centred approach prevailed, more recently it has been proposed that fatty acids are an important potential source of the aetiological factor. In addition to elevated fasting glucose concentrations, the insulin-resistant subject displays an imbalance in fatty acid metabolism reflected by increased levels of circulating non-esterified fatty acids (NEFA) or free fatty acids (FFA) and TAG. This metabolic phenotype is exacerbated in the obese state [51] 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 [52]. 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 NEFA occurs, which leads to an imbalance between the oxidation of fat and of glucose (Fig. 1) [53]. 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 [54]. Shulman and colleagues [55] have challenged Randle's conventional hypothesis for the effects of fatty acids on insulin sensitivity using NMR. They have found that an increase in plasma FFA inhibits glucose transport and/or phosphorylation causing a reduction in glucose oxidation and muscle glycogen synthesis [56]. This is somewhat different to Randle's theory of the development of insulin resistance.

2.2 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, polygenetic, diet-related diseases including obesity, insulin resistance 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 in general.

For example, the "thrifty genotype" [57], which conferred a protective effect in times of food deprivation, promoting fat deposition, has now been proposed to explain the escalating incidence of obesity in recent times as food is

more abundant and physical activity has decreased. Insulin affects the transcription of several genes involved in energy homeostasis, glucose and lipid metabolism. Therefore, the interactions between genes, dietary fat composition and insulin sensitivity are the focus of many studies to define the molecular mechanisms that affect insulin responsive genes. One such gene is peroxisome proliferator-activated receptor gamma (PPARγ), which has shown strong association with the development of the metabolic syndrome and T2DM. PPARs are members of the nuclear-hormone receptor superfamily of which there are three isoforms PPARα, PPARβ/δ and PPARγ. Unsaturated fatty acids can bind to specific isoforms and activate PPARs, varying to the PPARs affinity depending on fatty acid chain length and degree of unsaturation. It has been demonstrated that several PUFAs [α-linoleic acid C18:2 n-6; γ-linolenic C18:3 n-6; arachidonic acid C20:4 n-6 and eicosapentaenoic acid (EPA) C20:5 n-3] activate PPARγ [58]. In the context of insulin resistance, PPARγ regulates adipogenesis and is involved in insulin sensitisation [58]. PPARγ promotes the storage of fat and increases adipocyte differentiation and enhances the transcription of genes important for lipogenesis [59].

The PPARγ Pro12Ala polymorphism has also been studied in context of gene-nutrient interactions as a candidate genetic marker relevant to insulin resistance and the metabolic syndrome. This variant results from a Pro to Ala substitution at the codon 12, which modulates the transcriptional activity of the gene. In a prospective population-based cohort study, Luan *et al.* (2001) demonstrated an important interaction between background dietary fat composition and the PPARγ Pro12Ala single nucleotide polymorphism. With an increase in the polyunsaturated to saturated ratio, an inverse relationship for BMI and insulin concentrations in Ala carriers but not Pro homozygotes was seen [60]. This important interaction between dietary fatty acid intake and the Pro12Ala polymorphism of the PPARγ gene makes relevant the effects of diet on insulin resistance and other aspects of the complex metabolic syndrome. Luan's study is backed up by a meta-analysis [61] reporting an association between Ala12, insulin sensitivity and a decreased risk of T2DM. In the Quebec Family Study, total fat and SFA intakes correlated with BMI, abdominal adipose tissue area, waist circumference and fasting glucose levels in Pro12 homozygotes but not seen in carriers of the Ala allele. The PPARγ example demonstrates the significance of gene-nutrient interactions in modulating components of the metabolic syndrome. However, there is a need to further substantiate research in this area.

2.3 Insulin signalling – disruption with NEFA

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 [62]. 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). Activation of phosphatidylinositol 3-kinase (PI3K), instrumental in glucose transporter (Glut) 4 translocation to the plasma membrane follows, facilitating increased glucose transport into the cells [55]. Circulating FFA (malonyl CoA, long-chain acyl-CoA) reduce muscle glucose oxidation and glycogen synthesis by impairing glucose transport [63] and impeding insulin-mediated tyrosine phosphorylation of IRS-1 and IRS-1-associated PI3K activity, which is associated with a substantial increase in membrane-bound protein kinase C theta (PKCθ), a known serine kinase. LCacylCoA generates a pool of diacylglycerol, which may activate PKCθ. Phosphorylation of IRS-1 on a serine residue affects Glut 4 translocation to the cell surface [54]. A similar mechanism involving IRS-2 is suggested to occur in the liver [55]. As the IR is a membrane-bound protein and its function is membrane dependent, it follows on that changes in membrane lipid, induced by dietary fat, impacts on the function of the plasma membrane IR [64]. In isolated rat adipocytes, it has been demonstrated that a high polyunsaturated to saturated diet increased IR function and increased glucose oxidation and glucose transport [65]. A high-PUFA diet can also increase receptor tyrosine kinase activity [66]. Again these dietary effects may be further determined by the degree of unsaturation and chain length of the unsaturated fatty acids, another potential theme for further research.

2.4 FFA and insulin secretion

FFA or NEFA have an important stimulatory effect on glucose-stimulated insulin secretion (GSIS), by maintaining a basal rate of insulin secretion, thus regulating adipose tissue lipolysis. In the metabolic syndrome and T2DM, increased NEFA contribute to hyperinsulinaemia and ultimately overexposure results in β-cell damage. Prentki and Corkey [67] proposed that an elevation of the basal glucose level produces an increase in glucose-derived malonyl CoA within the β-cell. Increased levels of intracellular malonyl CoA inhibit the activity of carnitine palmitoyl transferase-1 (CPT-1) as described by McGarry *et al.* [68] in the detailed explanation of glucose-fatty acid metabolism. This in turn causes an increase in long-chain acyl CoA levels. The malonyl-CoA/CPT-1 interaction is central to GSIS highlighting the role of fatty acids in the process [54]. The insulinotrophic effects of FFA differ with respect to the level of saturation. In one study, endogenous circulating FFA were eliminated with the anti-lipolytic agent nicotinic acid and replaced with soybean oil (which is predominantly PUFA) or lard oil (a source of SFA). After a glucose challenge, the proportion of insulin secretion was far more effective after the SFA treatment [69]. The 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 [70]. This fat accumulation in the islets and its subsequent effects on GSIS provides purpose for further studies in humans.

2.5 Other mechanisms-brief mention

As the definitive factor in the new IDF criteria for the metabolic syndrome, central obesity is associated with insulin resistance. Increasingly, the effects of diet on body fat distribution are attracting much interest in an effort to understand the factors that are associated with insulin resistance. A study of the effects of three isocaloric diets: saturated fat rich, monounsaturated rich and carbohydrate rich showed that the MUFA-rich diet prevented central fat redistribution and decreased postprandial adiponectin expression induced by a carbohydrate-rich diet [71]. Adipose tissue is an important endocrine organ that secretes several inflammatory markers known as adipokines. Adipokines such as tumour necrosis factor-α enhance adipocyte lipolysis, increasing the secretion of fatty acids from adipose tissue (NEFA) indirectly stimulating gluconeogenesis in the liver and also through direct adverse effects on insulin-signalling pathways [72–74] by inhibiting autophosphorylation of tyrosine residues of the IR, promoting serine phosphorylation of IRS-1. Serine phosphorylation of IR in adipocytes follows, inhibiting tyrosine phosphorylation. Resistin and IL-6 also have effects on insulin-mediated glucose uptake and impede insulin signalling.

Glucose-independent insulinotrophic peptide (GIP) and glucagon-like peptide-1 (GLP-1) are important factors in postprandial insulin secretion. The intestinal hormone GLP-1 is secreted within the ileal L cell after ingestion of carbohydrate and fat. Fatty acids have been demonstrated to have stimulatory effects on the hormone with long-chain (>C16) MUFA directly stimulating the L cell [75].

3 MUFA and insulin sensitivity

An understanding of the mechanisms involved in the development of insulin resistance in relation to the type of fat in the diet has been the focus of many studies. Epidemiological and cohort studies suggest a detrimental effect of total fat consumption in particular saturated fat on insulin resistance and the development of T2DM [76–79]. A summary of the human intervention studies that investigated the effect of MUFA interventions on insulin and glucose metabolism is presented in Table 2. It clearly shows that diets rich in MUFA tend to improve insulin sensitivity [80, 81], however not all studies showed a positive effect [50, 82]. 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 [49, 83] and reduced glycaemic control. An interesting intervention study in patients with T2DM demonstrated that, a high fat, MUFA-enriched diet (33% of total energy) resulted in lower insulin requirements and lower plasma glucose concentration compared to a low-fat (25% total energy), high-carbohydrate diet (60% total energy) [84]. The positive effect may have been related to the reduced carbohydrate load, which would otherwise stress already damaged pancreatic β-cells. Nevertheless, subsequent studies have failed to show positive effects of dietary MUFA enrichment and carbohydrate modifications on fasting insulin in diabetics [85, 86] or on insulin sensitivity in healthy subjects [87]. Based on this research, the American Diabetes Association has recommended dividing 60–70% of energy between carbohydrate and MUFA, depending on individual assessments and establishing desirable goals [88, 89].

Epidemiological studies have generally found no association between MUFA intake and risk of T2DM [90–92]. However, in a review [44] of the roles and types of fat in the risk of T2DM, a study examining intakes of MUFA through the increased consumption of olive oil found an association with lower fasting plasma glucose concentration [93]. In a cross-sectional study of the general population in the Southeast of Spain, an oral glucose tolerance test (OGTT) in 538 subjects to calculate insulin resistance and β-cell function using HOMA showed a favourable relationship between MUFA intake and β-cell insulin secretion [94]. Studies that have shown a positive association between MUFA and insulin metabolism [95, 96] could be rationalised by the fact that MUFA is inversely correlated with saturated fat intake [97] in a typical westernised diet [44].

In general, dietary intervention studies have shown inconsistent results in relation to the effect of MUFA on insulin sensitivity. These discrepancies may be due to differences and/or insufficient cohort size, diverse clinical characteristics of the cohorts (healthy, impaired fasting glycaemia (IFG), metabolic syndrome, diabetes), the nature of the dietary fat modification (MUFA *vs.* PUFA; MUFA *vs.* SFA; MUFA *vs.* carbohydrate), study duration, measures of insulin sensitivity and inappropriate study design [47, 49, 50, 84, 86, 87, 98–100].

In the well-cited KANWU study, a large group ($n = 162$) of healthy individuals were randomised to receive an isoenergetic high-saturated fat or high-MUFA diet for 12 weeks. Within each group, a sub-sample was randomly selected to consume a fish oil (n-3 PUFA) supplement or placebo. Insulin sensitivity was measured by the frequently sampled IVGTT. This study demonstrated that the high-fat diet reduced insulin sensitivity by 10%. The difference between the two dietary fat composition groups was only apparent after adjustment for total fat intake. The beneficial effect of the MUFA diet on insulin sensitivity was only apparent in subjects with total dietary fat intake less than

Table 2. Summary of human studies investigating the role of MUFA on glucose and insulin action

Study	Population	Type of study	Type of diet	Outcome
Thomsen *et al.* (2003)	12 overweight T2DM 5 women and 7 men	Fat meals	High SFA *vs.* high MUFA meal	GLP-1 responses highest after olive oil meal
Vessby *et al.* (2001)	162 healthy subjects	Randomised Intervention	Isoenergetic diet; high SFA *vs.* high MUFA	MUFA diet improved IS (<37% energy from fat)
Parillo *et al.* (1992)	10 NIDDM patients	Randomised crossover	High MUFA/low CH *vs.* High carbohydrate/low MUFA	High MUFA/low carbohydrate- ↓in postprandial glucose & plasma insulin
Sarkkinen *et al.* (1996)	31subjects IGT	Randomised Intervention	High fat MUFA rich *vs.* low fat PUFA enriched	MUFA rich diet- ↑ fasting plasma glucose S_G higher in MONO group
Garg *et al.* (1988)	10 NIDDM	Randomised crossover	High carbohydrate diet *vs.* high MUFA	High MONO diet-↑plasma glucose levels, ↑insulin requirements-improve glycaemic control
Perez-Jimenez *et al.* (2001)	59 young healthy subjects	Randomised crossover	Isoenergetic high MUFA *vs.* low fat High carbohydrate after a baseline saturated-fat	Improvement in IS with high-carbohydrate & high-MUFA diet
Uusitupa *et al.* (1994)	10 young healthy females	Randomised crossover	High SFA *vs.* high MUFA	MUFA diet-Glucose AUC lower;
Thomsen *et al.* (1999)	16 healthy (6 men, 10 women) (first degree relatives of T2DM)	Randomised crossover	Isocaloric carbohydrate rich or MUFA rich (olive oil)	IS similar
Marshall *et al.* (1997)	1069 individuals	Observational study	Habitual dietary intake	No associations with fasting insulin conc. for MUFA
Ryan *et al.* (2000)	11 T2DM	Observational	MUFA (oleic acid rich) *vs.* PUFA	MUFA ↑insulin resistance
Lovejoy *et al.* (2002)	25 healthy men and women	Randomised crossover	MUFA *vs* SFA *vs. Trans*	MUFA-no impact on IS
Esposito *et al.* (2004)	180 patients with MS (ATPIII)	Observational	Mediterranean style diet *vs.* Prudent diet	Med diet ↑ insulin resistance
Thomsen *et al.* (1999)	10 healthy people	Fat meals	High fat, high MUFA, control	GLP-1 and GIP responses↓after the olive oil meal
Mayer Davis *et al.* (1997)	1173 men and women, no diabetes	Cross-sectional	Habitual dietary intake	Higher fat intake associated with lower IS among obese; results consistent for MUFA
Mayer Davis *et al.* (1993)	544 non-diabetic twins	Cross-sectional	Habitual dietary intake	Higher intakes of SFA, oleic acid and linoleic acid-positively related to higher fasting insulin values
Rojo-Martinez *et al.* (2006)	538 free-living	Cross-sectional	Habitual dietary intake	Favourable relationship of MUFA with β-cell insulin secretion
Bonanome *et al.* (1991)	19 NIDDM patients	Intervention	3 isocaloric diet changes;	No significant changes noted for plasma glucose Phase 1&3 rich in carbohydrate, phase 2 rich in MUFA
Soriguer *et al.* (2004)	538 Pizarra Spain	Cross-sectional study	Habitual dietary intake; usual cooking oil used	IR less in people who used olive oil
Feskens *et al.* (1995)	338(Seven Countries Study)	Longitudinal	Habitual dietary intake	Intake of total, SFA, mono, dietary cholesterol higher in men with newly diagnosed diabetes
Salmeron *et al.* (2001)	84204 women	Prospective study	Habitual dietary intake	MUFA not associated with diabetes (Nurses Health Study) no diabetes
Trevisian *et al.* (1990)	4093 Italian men and women	Cross-sectional	Habitual dietary intake	Consumption of olive oil-inversely associated with glucose levels
Maron *et al.* (1991)	215non-diabetic men with CAD	Intervention	Habitual dietary intake	MUFA correlated positively with fasting insulin
Soriguer *et al.* (2006)	538 Pizarra Spain	Cross-sectional	Habitual dietary intake-high in oleic acid	Interaction between dietary MUFA and the PPARG2 Ala-12 allele

T2DM, Type 2 Diabetes Mellitus; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; GLP-1, glucagon-like peptide-1; IS, insulin sensitivity; NIDDM, non-insulin dependent diabetes mellitus; ↑, increase; IGT, impaired glucose tolerance; S_G, glucose effectiveness; AUC, area under curve; MS, metabolic syndrome; ATP III, Adult Treatment Panel III; GIP, Glucose independent insulinotrophic peptide; ↓, decrease; hx, history; IR, insulin resistant; CAD, coronary artery disease.

37% of total energy [47]. This apparent interaction between dietary fat composition and total fat intake has been confirmed in a smaller randomised crossover intervention study in 59 young healthy subjects [87]. In this study, subjects were randomly assigned to a carbohydrate-rich and MUFA-rich diets for 28 days after an initial high-saturated fat run-in period. Both the carbohydrate and MUFA-rich diets improved insulin sensitivity *in vivo*. In contrast, Lovejoy *et al*. [50] failed to demonstrate any positive effect of MUFA in a crossover study that compared the effects of MUFA, SFA and *trans*-fatty acid diets. Even so, an interesting *post-hoc* analysis demonstrated that insulin sensitivity was lower in overweight subjects after the SFA diet was compared to the MUFA diet when the cohort was stratified according to BMI. Therefore, it is fair to surmise that habitual dietary fat quantity and BMI status are important considerations that interact with dietary fat composition and may affect insulin dynamics in humans.

3.1 Olive oil-derived MUFA and insulin sensitivity

In 1997, a group of nutrition, medical and public health specialists gathered in Rome to reach a health consensus on olive oil and the Mediterranean diet. A working definition of the traditional Mediterranean diet was described: "The traditional (European) Mediterranean diet is characterised by an abundance of plant foods such as bread, pasta, vegetables, salad, legumes, fruit, nuts; olive oil as the principal source of fat; low to moderate amounts of fish, poultry, dairy products and eggs; only little amounts of red meat; low to moderate amounts of wine, normally consumed with meals. This diet is low in saturated fatty acids, rich in carbohydrates and fibre, and has a high content of monounsaturated fatty acids. These are primarily derived from olive oil" [101].

In a lead review article, Trichopoulou and Lagiou [102] consider the broader aspects of the Mediterranean diet in the different regions of the Mediterranean basin, but describe the commonality of the various diets being the fact that olive oil occupies a central position in all of them. Olive oil is used liberally in food preparation, cooking and serving and therefore is consumed in significant quantities in the Mediterranean regions. Although this raises the total fat quantity, the ratio of monounsaturated to saturated fat is higher than in other parts of the world [102] and the source of monounsaturated fat is predominantly derived from olive oil [103, 104].

The effect of the Mediterranean diet in particular, on different components of the metabolic syndrome, has been studied in detail. Improvements in endothelial function and a reduction in markers of systemic vascular inflammation and coagulation have been observed [105, 106]. In addition, the Mediterranean diet has significant beneficial effects on both systolic and diastolic blood pressure [107, 108], which corroborates the fact that many of the risk factors of the metabolic syndrome are indeed modifiable. Replacing saturated fat with monounsaturated fat reduces LDL cholesterol without a reduction in HDL cholesterol or an increase in TAG concentrations [109].

Olive oil, as a single component, has been studied in less detail; however, its role in being a major constituent of the Mediterranean diet leads many authors to conclude that the beneficial effects observed are attributable to this MUFA-rich source. In a cross-sectional study in the South of Spain, levels of insulin resistance were found to be lower in people who used olive oil than in those who used sunflower oil [81]. To ascertain the effects of a MUFA-rich fat meal, or diet on glycaemic control, lipoprotein metabolism or endothelial dysfunction, olive oil has been used to increase the MUFA content and its percentage contribution to energy.

Olive oil is rich in antioxidant and anti-thrombotic agents, which confer its protective effect against CVD and certain cancers [110]. Olive oil is comprised of 55–85% oleic acid, antioxidants such as vitamin E and phenolic compounds (hydroxytyrosol, tyrosol, oleuropein and verbacosid) [111]. Oleic acid (cis C18:1 n-9) makes up 92% of the MUFA present in foods, and almost 60–80% of oleic acid intake comes from olive oil. Oleic acid is well absorbed in humans [112]. The function of the phenols are important in the milieu of insulin resistance and the development of the metabolic syndrome in that they inhibit LDL cholesterol oxidation, platelet aggregation and thromboxane production, they stimulate anti-inflammatory components and increase nitric oxide production [103]. Many studies have used oleic acid-rich diets to examine the effects on insulin resistance. Ryan *et al*. [80] examined the relationship between change in membrane fatty acid composition and glucose transport as an index of insulin sensitivity and found a reduction in insulin resistance when a polyunsaturated (linoleic acid; C18:2 n-6) rich diet was changed to an oleic acid (C18:1 n-9) rich diet. This improvement was related to the change in membrane fluidity, as an oleic acid-rich membrane would be less fluid to its linoleic acid-rich equivalent. Referring back to the earlier discussion on the effects of fatty acids on GSIS, the structural difference in oleic acid (one-double bond) could lead to a stimulated release of insulin from the β-cell in response to glucose. In terms of gene-nutrient interactions high intakes of oleic acid can interact with polymorphisms of the PPARγ2 gene (Pro12Ala), resulting in increased insulin sensitivity [113]. In lean Zucker male rats exposed to an olive oil-rich MUFA or coconut oil-rich SFA diet, a greater stimulation of GLP-1 by GIP was observed with the oleic acid, while a blunting in the GLP-1 secretion induced by GIP was seen with palmitic acid [114]. In overweight T2DM patients and in healthy subjects, olive oil induced higher concentrations of GLP-1 further strengthening the relationship between fatty acid composition and incretin responses [115, 116].

3.2 Other dietary fats – Effects on insulin sensitivity

There are many intervention studies that have determined the effect of total fat intake on insulin sensitivity as measured by the clamp technique or variations of the frequently sampled IVGTT and shown that high-fat diets (>38% energy from fat) exacerbates insulin resistance. Low-fat diets, however, have been shown to have a beneficial effect [82, 117–119]. Total fat has been shown to be inversely related to insulin sensitivity irrespective of the type of fat, especially among the obese, an already vulnerable group in terms of increased risk of the metabolic syndrome and T2DM. An *ad libitum*, low-fat, high-fibre diet has been shown to encourage weight loss in patients with T2DM without the adverse effects on plasma lipids or on glycaemic control [120]. Categorising people by BMI has shown an increase in insulin sensitivity in the more overweight and obese categories when total dietary fat is reduced [50]. The use of 24-h 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 [121] found higher proportions of SFA in non-insulin-dependent diabetes mellitus patients as compared with healthy controls. In a later study, Vessby *et al*. [122] measured fatty acid composition of serum cholesterol esters, representative of the dietary fat compositions for the previous 6–8 weeks in 70-year-old men. Insulin sensitivity was associated with low proportions of palmitic (C16:0) and palmitoloeic (C16:1 n-7) acids and a high proportion of linoleic acid (C18:2 n-6). The fatty acids in serum lipids in the insulin-resistant subjects suggest possible changes in the activities of desaturation (decrease in Δ5 desaturase, increase in Δ6 and Δ9 desaturase) and elongation enzymes [121], suggesting that these enzymes are insulin dependent. The desaturase activities have also been observed with obesity and lifestyle factors in men and women [123].

The anti-atherogenic effects of linoleic acid are well documented [124]. Effects on insulin sensitivity are less clear cut, however. *In vitro* and animal studies have shown encouraging effects of PUFA on insulin action [125], especially with fish oils of which long-chain n-3 PUFA such as EPA and docosahexanoic acid (DHA) are found in high concentrations [126]. Laaksonen *et al.* [127] showed an association of the n-6 PUFA linoleate in serum with the risk for development of IFG or diabetes in middle-aged men over a 4-year follow up. Salmeron *et al*. [92] postulated a reduction in T2DM by replacing 2% of energy from *trans* fatty acids isoenergetically with PUFA, indicating that increased intakes of long-chain PUFA reduce insulin resistance. Animal studies have confirmed this, showing significant decreases in lipids, glucose levels and an increase in insulin sensitivity [128]. Considering the nutritional determinants of dietary fats on insulin sensitivity, a long-chain n-3 PUFA (EPA and DHA)-rich diet fed to rats prevented insulin resistance in muscle and liver, through the hypothesised effect of reducing fat content in muscle and thus maintaining normal PI3K activity and expression and translocation of Glut 4 receptors and by inhibiting hepatic glucose production in the liver [41]. Intake of fish oils directly influences the fatty acid composition of membrane phospholipids [126] and increases insulin sensitivity [129, 130]. Effects of n-3 PUFA requires sufficient duration to permit a change in the component of the cell membrane phospholipids to assess their direct action on insulin resistance in contrast to their established indirect effect on endothelial function, platelet aggregation, TAG concentration and arrhythmic effects [131]. Epidemiological data confirming the effects of fish and fish oil supplements on insulin sensitivity is not a wealthy resource. The Seven Countries Study and the Nurses Health Study showed inverse associations between fish consumption and glucose concentrations and the incidence of T2DM [91, 92]. However, other studies have failed to replicate these findings [132]. In the KANWU study, supplementation with n-3 PUFA did not influence insulin sensitivity on the high-fat or high-monounsaturated fat diets, confirming the controversial effects of n-3 PUFA in human interventions as opposed to animal models.

Although the effects of *trans* fatty acids on lipoprotein concentrations (increase in LDL cholesterol, decrease in HDL cholesterol) are well publicised, they could also affect insulin sensitivity through the inhibition of desaturase enzymes [44]. The Nurses Health Study was paramount in highlighting the detrimental effects of *trans* fatty acids on human health in terms of development of T2DM. An increase of 2% energy from *trans* fatty acids was associated with a 39% increase in diabetes risk [92].

4 Perspectives for the future

The optimal amount and type of dietary fat for the prevention of insulin resistance and the metabolic syndrome remains unclear. Despite a robust inverse association between the overall Mediterranean diet score and mortality, no appreciable associations were seen for most of the individual dietary components including olive oil, which would suggest that the cumulative effects (synergistic or interactive) of multiple dietary components may be substantial. In other words, the effect appears to be more than the sum of its parts [133]. A study of the Catalan population in Spain [103] demonstrated that higher intakes of olive oil were associated with higher total fat intakes and had more ideal food patterns such as increase fish, eggs and vegetable intakes.

From the abundance of figures and statistics in the literature of the rising rates and prevalence of insulin-resistant states such as obesity T2DM and CVD, it is clear that the simple equation of energy expenditure greater than energy intake will lead to weight loss is not a clear and effective message. While weight loss is advocated by medics, dietitians and researchers as the primary goal to moderate the abnormalities associated with insulin resistance for so many overweight and obese, it is a futile effort. However, as presented in this review through animal and human studies, changing the macronutrient content of isocaloric diets reveals positive biochemical outcomes.

LIPGENE is an EU Sixth Framework Programme Integrated Project (2004 – 2009) entitled "Diet, genomics and the metabolic syndrome: an integrated nutrition, agro-food, social and economic analysis". LIPGENE aims to study the effect of dietary fat quantity and quality and of diet-sensitive genotypes on insulin sensitivity and other risk markers of the metabolic syndrome (www.lipgene.tcd.ie). A human dietary intervention study carried out in 8 European countries will determine the impact of dietary fat on characteristics of the metabolic syndrome and is fundamental to the LIPGENE project. Current dietary fat recommendations state total fat should comprise <30% of total energy, of which 10 – 15% should be monounsaturated fat. LIPGENE will investigate the substitution of modifiable fat sources used in every day life such a margarines, oils and baking fats with MUFA equivalent products as a vehicle to deliver an increased source to people who would otherwise not consume food sources rich in this nutrient. An animal nutrition component of LIPGENE will focus on altering the fatty acid composition of meat and milk through the manipulation of cattle's diets. Typically, 70 – 75% of the fatty acids present in milk are SFA, 20 – 25% are MUFA, the remainder PUFA. It is possible to reduce the amount of C16 – 18 SFA and increase the amount of MUFA (as oleic acid; C18:1 n-9) present in milk by increasing the supply of SFA of 18-carbon chain length, or greater, to the mammary gland, as stearic acid can be desaturated within it [134]. With the planning of controlled studies and the development of dietary recommendations, the current guidelines need to be considered, especially in view of the fact in Mediterranean regions, a higher percentage of total fat is consumed, albeit, monounsaturated predominantly, therefore, advising a low fat diet with <30% energy as fat would result in a drastic reduction in olive oil consumption. In recent studies [135, 136], changes in the Mediterranean diet have been observed, such that a move in the direction of 'modernisation' is occurring leaving the traditional Mediterranean diet of high olive oil intakes in decline. From the evidence presented here, a shift back to the traditional Mediterranean diet should be advocated. Many prospective studies need to be undertaken to confirm the effects of varying macronutrients in the diets and their role in insulin resistance to unravel the effects of the different fatty acids on this important causative factor of the metabolic syndrome and T2DM. From this, another question arises; to assist in effective clinical diagnosis and policy developments or in designing dietary prevention programmes, should all definitions of the metabolic syndrome incorporate insulin resistance as a main feature?

5 References

[1] Berson, S. A., Yallow, R. S., Insulin 'antagonists' and insulin resistance, in: Ellenberg, M., Rifkin, H. (Eds.), *Diabetes Mellitus: Theory and Practice*, McGraw Hill, New York 1970, pp. 388 – 423.

[2] Reaven, G., Banting Lecture 1988. Role of insulin resistance in human disease. *Diabetes* 1988, *37*, 1595 – 1607.

[3] Reaven, G. M., The metabolic syndrome: is this diagnosis necessary? *Am. J. Clin. Nutr.* 2006, *83*, 1237 – 1247.

[4] Bonora, E., Kiechl, S., Willeit, J., Oberhollenzer, F., *et al*., Prevalence of insulin resistance in metabolic disorders: the Bruneck Study. *Diabetes* 1998, *47*, 1643 – 1649.

[5] Zethelius, B., Hales, C., Berne, C., Insulin resistance, proinsulin and coronary heart disease. A population based, follow-up study using the euglycemic clamp. *Diabetologia* 2003, *46*, A76.

[6] Zimmet, P., Alberti, K. G., Shaw, J., Global and societal implications of the diabetes epidemic. *Nature* 2001, *414*, 782 – 787.

[7] Riccardi, G., Giacco, R., Rivellese, A. A., Dietary fat, insulin sensitivity and the metabolic syndrome. *Clin. Nutr.* 2004, *23*, 447 – 456.

[8] Parker, D. R., Weiss, S. T., Troisi, R., Cassano, P. A., *et al*., Relationship of dietary saturated fatty acids and body habitus to serum insulin concentrations: the Normative Aging Study. *Am. J. Clin. Nutr.* 1993, *58*, 129 – 136.

[9] Clausen, J. O., Borch-Johnsen, K., Ibsen, H., Bergman, R. N., *et al*., Insulin sensitivity index, acute insulin response, and glucose effectiveness in a population-based sample of 380 young healthy Caucasians. Analysis of the impact of gender, body fat, physical fitness, and life-style factors. *J. Clin. Invest.* 1996, *98*, 1195 – 1209.

[10] Albu, J., Konnarides, C., Pi-Suniyer, F., Weight Control: metabolic and cardiovascular effects. *Diabetes Rev.* 1995, *3*, 335 – 347.

[11] Cummings, D. E., Schwartz, M. W., Genetics and pathophysiology of human obesity. *Annu. Rev. Med.* 2003, *54*, 453 – 471.

[12] Monzillo, L. U., Hamdy, O., Evaluation of insulin sensitivity in clinical practice and in research settings. *Nutr. Rev.* 2003, *61*, 397 – 412.

[13] DeFronzo, R. A., Tobin, J. D., Andres, R., Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am. J. Physiol.* 1979, *237*, E214 – 223.

[14] Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A. *et al*., Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985, *28*, 412 – 419.

[15] Wallace, T. M., Matthews, D. R., The assessment of insulin resistance in man. *Diabet. Med.* 2002, *19*, 527 – 534.

[16] Beard, J. C., Bergman, R. N., Ward, W. K., Porte, D. Jr., The insulin sensitivity index in nondiabetic man. Correlation between clamp-derived and IVGTT-derived values. *Diabetes* 1986, *35*, 362 – 369.

[17] Pacini, G., Bergman, R. N., MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. *Comput. Methods Programs Biomed.* 1986, *23*, 113 – 122.

[18] Boston, R. C., Stefanovski, D., Moate, P. J., Sumner, A. E., *et al*., MINMOD Millennium: a computer program to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test. *Diabetes Technol. Ther.* 2003, *5*, 1003 – 1015.

[19] Bergman, R. N., Finegood, D. T., Ader, M., Assessment of insulin sensitivity *in vivo*. *Endocr. Rev.* 1985, *6*, 45 – 86.

[20] Mather, K. J., Hunt, A. E., Steinberg, H. O., Paradisi, G., *et al*., Repeatability characteristics of simple indices of insulin resistance: implications for research applications. *J. Clin. Endocrinol. Metab.* 2001, *86*, 5457 – 5464.

[21] Levy, J. C., Matthews, D. R., Hermans, M. P., Correct homeostasis model assessment (HOMA) evaluation uses the computer program. *Diabetes Care* 1998, *21*, 2191 – 2192.

[22] Katz, A., Nambi, S. S., Mather, K., Baron, A. D., *et al*., Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J. Clin. Endocrinol. Metab.* 2000, *85*, 2402 – 2410.

[23] Alberti, K. G., Zimmet, P. Z., Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet. Med.* 1998, *15*, 539 – 553.

[24] Executive summary of the Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 2001, *285*, 2486 – 2497.

[25] Shafrir, E., Development and consequences of insulin resistance: lessons from animals with hyperinsulinaemia. *Diabetes Metab.* 1996, *22*, 122 – 131.

[26] Zimmet, P., Boyko, E. J., Collier, G. R., de Courten, M., Etiology of the metabolic syndrome: potential role of insulin resistance, leptin resistance, and other players. *Ann. N. Y. Acad. Sci.* 1999, *892*, 25 – 44.

[27] Hanley, A. J., Wagenknecht, L. E., D'Agostino, R. B., Jr., Zinman, B., Haffner, S. M., Identification of subjects with insulin resistance and beta-cell dysfunction using alternative definitions of the metabolic syndrome. *Diabetes* 2003, *52*, 2740 – 2747.

[28] Karter, A. J., Mayer-Davis, E. J., Selby, J. V., D'Agostino, R. B. Jr. *et al*., Insulin sensitivity and abdominal obesity in African-American, Hispanic, and non-Hispanic white men and women. The Insulin Resistance and Atherosclerosis Study. *Diabetes* 1996, *45*, 1547 – 1555.

[29] Howard, B. V., Mayer-Davis, E. J., Goff, D., Zaccaro, D. J., *et al*., Relationships between insulin resistance and lipoproteins in nondiabetic African Americans, Hispanics, and non-Hispanic whites: the Insulin Resistance Atherosclerosis Study. *Metabolism* 1998, *47*, 1174 – 1179.

[30] Eckel, R. H., Grundy, S. M., Zimmet, P. Z., The metabolic syndrome. *Lancet* 2005, *365*, 1415 – 1428.

[31] Athyros, V. G., Ganotakis, E. S., Elisaf, M., Mikhailidis, D. P., The prevalence of the metabolic syndrome using the National Cholesterol Educational Program and International Diabetes Federation definitions. *Curr. Med. Res. Opin.* 2005, *21*, 1157 – 1159.

[32] Lawlor, D. A., Smith, G. D., Ebrahim, S., Does the new International Diabetes Federation definition of the metabolic syndrome predict CHD any more strongly than older definitions? Findings from the British Women's Heart and Health Study. *Diabetologia* 2006, *49*, 41 – 48.

[33] Sandhofer, A., Iglseder, B., Paulweber, B., Ebenbichler, C. F., Patsch, J. R., Comparison of different definitions of the metabolic syndrome. *Eur. J. Clin. Invest.* 2007, *37*, 109 – 116.

[34] Balkau, B., Charles, M. A., Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR). *Diabet. Med.* 1999, *16*, 442 – 443.

[35] Ford, E. S., Giles, W. H., Dietz, W. H., Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA* 2002, *287*, 356 – 359.

[36] King, H., Aubert, R. E., Herman, W. H., Global burden of diabetes, 1995 – 2025: prevalence, numerical estimates, and projections. *Diabetes Care* 1998, *21*, 1414 – 1431.

[37] Sunehag, A. L., Toffolo, G., Campioni, M., Bier, D. M., Haymond, M. W., Effects of dietary macronutrient intake on insulin sensitivity and secretion and glucose and lipid metabolism in healthy, obese adolescents. *J. Clin. Endocrinol. Metab.* 2005, *90*, 4496 – 4502.

[38] Keys, A., *Seven Countries: A multivariate Analysis of Death and Coronary Heart Disease*, Harvard University Press, Cambridge 1980.

[39] de Lorgeril, M., Salem, P., Martin, J. L., Monjaud, I., *et al*., Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. *Circulation* 1999, *99*, 779 – 785.

[40] EUROASPIRE., A European Society of Cardiology survey of secondary prevention of coronary heart disease: principal results. EUROASPIRE Study Group. European action on secondary prevention through intervention to reduce events. *Eur. Heart J.* 1997, *18*, 1569 – 1582.

[41] Taouis, M., Dagou, C., Ster, C., Durand, G., Pinault, M., Delarue, J., N-3 polyunsaturated fatty acids prevent the defect of insulin receptor signaling in muscle. *Am. J. Physiol. Endocrinol. Metab.* 2002, *282*, E664 – 671.

[42] Luo, J., Rizkalla, S. W., Boillot, J., Alamowitch, C., *et al*., Dietary (n-3) polyunsaturated fatty acids improve adipocyte insulin action and glucose metabolism in insulin-resistant rats: relation to membrane fatty acids. *J. Nutr.* 1996, *126*, 1951 – 1958.

[43] Hunnicutt, J. W., Hardy, R. W., Williford, J., McDonald, J. M., Saturated fatty acid-induced insulin resistance in rat adipocytes. *Diabetes* 1994, *43*, 540 – 545.

[44] Hu, F. B., van Dam, R. M., Liu, S., Diet and risk of Type II diabetes: the role of types of fat and carbohydrate. *Diabetologia* 2001, *44*, 805 – 817.

[45] Berry, E. M., Who's afraid of n-6 polyunsaturated fatty acids? Methodological considerations for assessing whether they are harmful. *Nutr. Metab. Cardiovasc. Dis.* 2001, *11*, 181 – 188.

[46] Roche, H. M., Phillips, C., Gibney, M. J., The metabolic syndrome: the crossroads of diet and genetics. *Proc. Nutr. Soc.* 2005, *64*, 371 – 377.

[47] Vessby, B., Unsitupa, M., Hermansen, K., Riccardi, G., *et al*., Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. *Diabetologia* 2001, *44*, 312 – 319.

[48] Salas, J., Lopez Miranda, J., Jansen, S., Zambrana, J. L., *et al*., [The diet rich in monounsaturated fat modifies in a beneficial way carbohydrate metabolism and arterial pressure]. *Med. Clin. (Barc)* 1999, *113*, 765 – 769.

[49] Parillo, M., Rivellese, A. A., Ciardullo, A. V., Capaldo, B., *et al*., A high-monounsaturated-fat/low-carbohydrate diet improves peripheral insulin sensitivity in non-insulin-dependent diabetic patients. *Metabolism* 1992, *41*, 1373 – 1378.

[50] Lovejoy, J. C., Smith, S. R., Champagne, C. M., Most, M. M., *et al*., Effects of diets enriched in saturated (palmitic), monounsaturated (oleic), or trans (elaidic) fatty acids on insulin sensitivity and substrate oxidation in healthy adults. *Diabetes Care* 2002, *25*, 1283 – 1288.

[51] Boden, G., Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes* 1997, *46*, 3 – 10.

[52] Reaven, G. M., Hollenbeck, C., Jeng, C. Y., Wu, M. S., Chen, Y. D., Measurement of plasma glucose, free fatty acid, lactate, and insulin for 24 h in patients with NIDDM. *Diabetes* 1988, *37*, 1020 – 1024.

[53] Randle, P. J., Garland, P. B., Hales, C. N., Newsholme, E. A., The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* 1963, *1*, 785 – 789.

[54] McGarry, J. D., Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. *Diabetes* 2002, *51*, 7 – 18.

[55] Shulman, G. I., Cellular mechanisms of insulin resistance. *J. Clin. Invest.* 2000, *106*, 171 – 176.

[56] Roden, M., Price, T. B., Perseghin, G., Peterson, K. F., *et al*., Mechanism of free fatty acid-induced insulin resistance in humans. *J. Clin. Invest.* 1996, *97*, 2859 – 2865.

[57] Neel, J. V., Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? *Am. J. Hum. Genet.* 1962, *14*, 353 – 362.

[58] Daynes, R. A., Jones, D. C., Emerging roles of PPARs in inflammation and immunity. *Nat. Rev. Immunol.* 2002, *2*, 748 – 759.

[59] Desvergne, B., Wahli, W., Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr. Rev.* 1999, *20*, 649 – 688.

[60] Luan, J., Browne, P. O., Harding, A. H., Halsall, D. J., *et al*., Evidence for gene-nutrient interaction at the PPARgamma locus. *Diabetes* 2001, *50*, 686 – 689.

[61] Altshuler, D., Hirschhorn, J. N., Klannemark, M., Lindgren, C. M., *et al*., The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat. Genet.* 2000, *26*, 76 – 80.

[62] Hotamisligil, G. S., Peraldi, P., Budavari, A., Ellis, R., *et al*., IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science* 1996, *271*, 665 – 668.

[63] Griffin, M. E., Marcucci, M. J., Cline, G. W., Bell, K., *et al*., Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade. *Diabetes* 1999, *48*, 1270 – 1274.

[64] Gletsu, N. A., Clandinin, M. T., Impact of dietary fatty acid composition on insulin action at the nucleus. *Ann. N. Y. Acad. Sci.* 1997, *827*, 188 – 199.

[65] Field, C. J., Toyomizu, M., Clandinin, M. T., Relationship between dietary fat, adipocyte membrane composition and insulin binding in the rat. *J. Nutr.* 1989, *119*, 1483 – 1489.

[66] Fickova, M., Hubert, P., Klimes, I., Staedel, C., *et al*., Dietary fish oil and olive oil improve the liver insulin receptor tyrosine kinase activity in high sucrose fed rats. *Endocr. Regul.* 1994, *28*, 187 – 197.

[67] Prentki, M., Corkey, B. E., Are the beta-cell signaling molecules malonyl-CoA and cystolic long-chain acyl-CoA implicated in multiple tissue defects of obesity and NIDDM? *Diabetes* 1996, *45*, 273 – 283.

[68] McGarry, J. D., Mannaerts, G. P., Foster, D. W., A possible role for malonyl-CoA in the regulation of hepatic fatty acid oxidation and ketogenesis. *J. Clin. Invest.* 1977, *60*, 265 – 270.

[69] Stein, D. T., Esser, V., Stevenson, B. E., Lane, K. E., *et al*., Essentiality of circulating fatty acids for glucose-stimulated insulin secretion in the fasted rat. *J. Clin. Invest.* 1996, *97*, 2728 – 2735.

[70] Shimabukuro, M., Zhou, Y. T., Levi, M., Unger, R. H., Fatty acid-induced beta cell apoptosis: a link between obesity and diabetes. *Proc. Natl. Acad. Sci. USA* 1998, *95*, 2498 – 2502.

[71] Paniagua, J. A., Gallego de la Sacristana, A., Romero, I., Vidal-Puig, A. *et al*., MUFA-rich diet prevents central body fat distributon and decreases postprandial adiponectin expression induced by a carbohydrate rich diet in insulin-resistant subjects. *Diabetes Care* 2007, *30*, 1717 – 1723.

[72] Ahima, R. S., Central actions of adipocyte hormones. *Trends Endocrinol. Metab.* 2005, *16*, 307 – 313.

[73] Frayn, K. N., Visceral fat and insulin resistance – causative or correlative? *Br. J. Nutr.* 2000, *83 (Suppl 1)*, S71 – 77.

[74] Itani, S. I., Ruderman, N. B., Schmieder, F., Boden, G., Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IkappaB-alpha. *Diabetes* 2002, *51*, 2005 – 2011.

[75] Rocca, A. S., Brubaker, P. L., Stereospecific effects of fatty acids on proglucagon-derived peptide secretion in fetal rat intestinal cultures. *Endocrinology* 1995, *136*, 5593 – 5599.

[76] van Dam, R. M., Willett, W. C., Rimm, E. B., Stampfer, M. J., Hu, F. B., Dietary fat and meat intake in relation to risk of type 2 diabetes in men. *Diabetes Care* 2002, *25*, 417 – 424.

[77] West, K. M., Kalbfleisch, J. M., Influence of nutritional factors on prevalence of diabetes. *Diabetes* 1971, *20*, 99 – 108.

[78] Wang, L., Folsom, A. R., Zheng, Z. J., Pankow, J. S., Eckfeldt, J. H., Plasma fatty acid composition and incidence of diabetes in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. *Am. J. Clin. Nutr.* 2003, *78*, 91 – 98.

[79] Meyer, K. A., Kushi, L. H., Jacobs, D. R. Jr., Folsom, A. R., Dietary fat and incidence of type 2 diabetes in older Iowa women. *Diabetes Care* 2001, *24*, 1528 – 1535.

[80] Ryan, M., McInerney, D., Owens, D., Collins, P., *et al*., Diabetes and the Mediterranean diet: a beneficial effect of oleic acid on insulin sensitivity, adipocyte glucose transport and endothelium-dependent vasoreactivity. *QJM* 2000, *93*, 85 – 91.

[81] Soriguer, F., Esteva, I., Rojo-Martinez, G., Ruiz de Adana, M. S. *et al*., Oleic acid from cooking oils is associated with lower insulin resistance in the general population (Pizarra study). *Eur. J. Endocrinol.* 2004, *150*, 33 – 39.

[82] Mayer-Davis, E. J., Monaco, J. H., Hoen, H. M., Carmichael, S. *et al*., Dietary fat and insulin sensitivity in a triethnic population: the role of obesity. The Insulin Resistance Atherosclerosis Study (IRAS). *Am. J. Clin. Nutr.* 1997, *65*, 79 – 87.

[83] Consensus Development Conference on Diet and Exercise in Non-Insulin-Dependent Diabetes Mellitus. National Institutes of Health. *Diabetes Care* 1987, *10*, 639 – 644.

[84] Garg, A., Bonanome, A., Grundy, S. M., Zhang, Z. J., Unger, R. H., Comparison of a high-carbohydrate diet with a high-monounsaturated-fat diet in patients with non-insulin-dependent diabetes mellitus. *N. Engl. J. Med.* 1988, *319*, 829 – 834.

[85] Garg, A., High-monounsaturated-fat diets for patients with diabetes mellitus: a meta-analysis. *Am. J. Clin. Nutr.* 1998, *67*, 577S – 582S.

[86] Bonanome, A., Visona, A., Lusiani, L., Beltramello, G., *et al*., Carbohydrate and lipid metabolism in patients with non-insulin-dependent diabetes mellitus: effects of a low-fat, high-carbohydrate diet vs a diet high in monounsaturated fatty acids. *Am. J. Clin. Nutr.* 1991, *54*, 586 – 590.

[87] Perez-Jimenez, F., Lopez-Miranda, J., Pinillos, M. D., Gomez, P., *et al*., A Mediterranean and a high-carbohydrate diet improve glucose metabolism in healthy young persons. *Diabetologia* 2001, *44*, 2038 – 2043.

[88] Evidence-Based Nutrition Principles and Recommendations for the Treatment and Prevention of Diabetes and Related Complications. *Diabetes Care* 2002, *25 (Suppl. 1)*, S50 – S60.

[89] Nutrition Recommendations and Interventions for Diabetes. A position statement of the American Diabetes Association. *Diabetes Care* 2007, *30*, S48 – S65.

[90] Colditz, G. A., Manson, J. E., Stampfer, M. J., Rosner, B., *et al*., Diet and risk of clinical diabetes in women. *Am. J. Clin. Nutr.* 1992, *55*, 1018 – 1023.

[91] Feskens, E. J., Virtanen, S. M., Rasanen, L., Tuomilehto, J., *et al*., Dietary factors determining diabetes and impaired glucose tolerance. A 20-year follow-up of the Finnish and Dutch cohorts of the Seven Countries Study. *Diabetes Care* 1995, *18*, 1104 – 1112.

[92] Salmeron, J., Hu, F. B., Manson, J. E., Stampfer, M. J., *et al*., Dietary fat intake and risk of type 2 diabetes in women. *Am. J. Clin. Nutr.* 2001, *73*, 1019 – 1026.

[93] Trevisan, M., Krogh, V., Freudenheim, J., Blake, A., *et al*., Consumption of olive oil, butter, and vegetable oils and coronary heart disease risk factors. The Research Group ATS-RF2 of the Italian National Research Council. *JAMA* 1990, *263*, 688 – 692.

[94] Rojo-Martinez, G., Esteva, I., Ruiz de Adana, M. S., Garcia-Almeida, J. M. *et al*., Dietary fatty acids and insulin secretion: a population-based study. *Eur. J. Clin. Nutr.* 2006, *60*, 1195 – 1200.

[95] Maron, D. J., Fair, J. M., Haskell, W. L., Saturated fat intake and insulin resistance in men with coronary artery disease. The Stanford Coronary Risk Intervention Project Investigators and Staff. *Circulation* 1991, *84*, 2020 – 2027.

[96] Mayer, E. J., Newman, B., Quesenberry, C. P. Jr., Selby, J. V., Usual dietary fat intake and insulin concentrations in healthy women twins. *Diabetes Care* 1993, *16*, 1459 – 1469.

[97] Marshall, J. A., Bessesen, D. H., Hamman, R. F., High saturated fat and low starch and fibre are associated with hyperinsulinaemia in a non-diabetic population: the San Luis Valley Diabetes Study. *Diabetologia* 1997, *40*, 430 – 438.

[98] Thomsen, C., Rasmussen, O., Christiansen, C., Pedersen, E. *et al*., Comparison of the effects of a monounsaturated fat diet and a high carbohydrate diet on cardiovascular risk factors in first degree relatives to type-2 diabetic subjects. *Eur. J. Clin. Nutr.* 1999, *53*, 818 – 823.

[99] Sarkkinen, E., Schwab, U., Niskanen, L., Hannuksela, M., *et al*., The effects of monounsaturated-fat enriched diet and polyunsaturated-fat enriched diet on lipid and glucose metabolism in subjects with impaired glucose tolerance. *Eur. J. Clin. Nutr.* 1996, *50*, 592 – 598.

[100] Uusitupa, M., Schwab, U., Makimattila, S., Karhapaa, P., *et al*., Effects of two high-fat diets with different fatty acid compositions on glucose and lipid metabolism in healthy young women. *Am. J. Clin. Nutr.* 1994, *59*, 1310 – 1316.

[101] Assmann, G., de Backer, G., Bagnara, S., Betteridge, J., *et al*., International consensus statement on olive oil and the Mediterranean diet: implications for health in Europe. The Olive Oil and the Mediterranean Diet Panel. *Eur. J. Cancer Prev.* 1997, *6*, 418 – 421.

[102] Trichopoulou, A., Lagiou, P., Healthy traditional Mediterranean diet: an expression of culture, history, and lifestyle. *Nutr. Rev.* 1997, *55*, 383 – 389.

[103] Serra-Majem, L., de la Cruz, J. N., Ribas, L., Salleras, L., Mediterranean diet and health: is all the secret in olive oil? *Pathophysiol. Haemost. Thromb.* 2003, *33*, 461 – 465.

[104] Trichopoulou, A., Katsouyanni, K., Gnardellis, C., The traditional Greek diet. *Eur. J. Clin. Nutr.* 1993, *47 (Suppl 1)*, S76 – 81.

[105] Esposito, K., Marfella, R., Ciotola, M., Di Palo, C. *et al*., Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. *JAMA* 2004, *292*, 1440 – 1446.

[106] Chrysohoou, C., Panagiotakos, D. B., Pitsavos, C., Das, U. N., Stefanadis, C., Adherence to the Mediterranean diet attenuates inflammation and coagulation process in healthy adults: The ATTICA Study. *J. Am. Coll. Cardiol.* 2004, *44*, 152 – 158.

[107] Psaltopoulou, T., Naska, A., Orfanos, P., Trichopoulos, D., *et al*., Olive oil, the Mediterranean diet, and arterial blood pressure: the Greek European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Am. J. Clin. Nutr.* 2004, *80*, 1012 – 1018.

[108] Panagiotakos, D. B., Polychronopoulos, E., The role of Mediterranean diet in the epidemiology of metabolic syndrome; converting epidemiology to clinical practice. *Lipids Health Dis.* 2005, *4*, 7.

[109] Ascherio, A., Epidemiologic studies on dietary fats and coronary heart disease. *Am. J. Med.* 2002, *113 (Suppl 9B)*, 9S – 12S.

[110] Keys, A., Mediterranean diet and public health: personal reflections. *Am. J. Clin. Nutr.* 1995, *61*, 1321S – 1323S.

[111] James, W. P., Duthie, G. G., Wahle, K. W., The Mediterranean diet: protective or simply non-toxic? *Eur. J. Clin. Nutr.* 1989, *43 (Suppl 2)*, 31 – 41.

[112] Jones, P. J., Pencharz, P. B., Clandinin, M. T., Absorption of ^{13}C-labeled stearic, oleic, and linoleic acids in humans: application to breath tests. *J. Lab. Clin. Med.* 1985, *105*, 647 – 652.

[113] Soriguer, F., Morcillo, S., Cardona, F., Rojo-Martinez, G., *et al*., Pro12Ala polymorphism of the PPARG2 gene is associated with type 2 diabetes mellitus and peripheral insulin sensitivity in a population with a high intake of oleic acid. *J. Nutr.* 2006, *136*, 2325 – 2330.

[114] Rocca, A. S., LaGreca, J., Kalitsky, J., Brubaker, P. L., Monounsaturated fatty acid diets improve glycemic tolerance through increased secretion of glucagon-like peptide-1. *Endocrinology* 2001, *142*, 1148 – 1155.

[115] Thomsen, C., Storm, H., Holst, J. J., Hermansen, K., Differential effects of saturated and monounsaturated fats on postprandial lipemia and glucagon-like peptide 1 responses in patients with type 2 diabetes. *Am. J. Clin. Nutr.* 2003, *77*, 605 – 611.

[116] Thomsen, C., Rasmussen, O., Lousen, T., Holst, J. J., *et al*., Differential effects of saturated and monounsaturated fatty acids on postprandial lipemia and incretin responses in healthy subjects. *Am. J. Clin. Nutr.* 1999, *69*, 1135 – 1143.

[117] Fukagawa, N. K., Anderson, J. W., Hageman, G., Young, V. R., Minaker, K. L., High-carbohydrate, high-fiber diets increase peripheral insulin sensitivity in healthy young and old adults. *Am. J. Clin. Nutr.* 1990, *52*, 524 – 528.

[118] Chen, M., Bergman, R. N., Porte, D. Jr., Insulin resistance and beta-cell dysfunction in aging: the importance of dietary carbohydrate. *J. Clin. Endocrinol. Metab.* 1988, *67*, 951 – 957.

[119] Lovejoy, J. C., Windhauser, M. M., Rood, J. C., de la Bretonne, J. A., Effect of a controlled high-fat versus low-fat diet on insulin sensitivity and leptin levels in African-American and Caucasian women. *Metabolism* 1998, *47*, 1520 – 1524.

[120] Gerhard, G. T., Ahmann, A., Meeuws, K., McMurry, M. P., *et al*., Effects of a low-fat diet compared with those of a high-monounsaturated fat diet on body weight, plasma lipids and lipoproteins, and glycemic control in type 2 diabetes. *Am. J. Clin. Nutr.* 2004, *80*, 668 – 673.

[121] Vessby, B., Dietary fat and insulin action in humans. *Br. J. Nutr.* 2000, *83 (Suppl 1)*, S91 – 96.

[122] Vessby, B., Tengblad, S., Lithell, H., Insulin sensitivity is related to the fatty acid composition of serum lipids and skeletal muscle phospholipids in 70-year-old men. *Diabetologia* 1994, *37*, 1044 – 1050.

[123] Warensjo, E., Ohrvall, M., Vessby, B., Fatty acid composition and estimated desaturase activities are associated with obesity and lifestyle variables in men and women. *Nutr. Metab. Cardiovasc. Dis.* 2006, *16*, 128 – 136.

[124] Heine, R. J., Mulder, C., Popp-Snijders, C., van der Meer, J., van der Veen, E. A., Linoleic-acid-enriched diet: long-term effects on serum lipoprotein and apolipoprotein concentrations and insulin sensitivity in noninsulin-dependent diabetic patients. *Am. J. Clin. Nutr.* 1989, *49*, 448 – 456.

[125] Storlien, L. H., Baur, L. A., Kriketos, A. D., Pan, D. A., *et al*., Dietary fats and insulin action. *Diabetologia* 1996, *39*, 621 – 631.

[126] Storlien, L. H., Jenkins, A. B., Chisholm, D. J., Pascoe, W. S., *et al*., Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and omega-3 fatty acids in muscle phospholipid. *Diabetes* 1991, *40*, 280 – 289.

[127] Laaksonen, D. E., Lakka, T. A., Lakka, H. M., Nyyssonen, K. *et al*., Serum fatty acid composition predicts development of impaired fasting glycaemia and diabetes in middle-aged men. *Diabet. Med.* 2002, *19*, 456 – 464.

[128] Somova, L., Moodley, K., Channa, M. L., Nadar, A., Dose-dependent effect of dietary fish-oil (n-3) polyunsaturated fatty acids on in vivo insulin sensitivity in rat. *Methods Find. Exp. Clin. Pharmacol.* 1999, *21*, 275 – 278.

[129] Pan, D. A., Lillioja, S., Milner, M. R., Kriketos, A. D., *et al*., Skeletal muscle membrane lipid composition is related to adiposity and insulin action. *J. Clin. Invest.* 1995, *96*, 2802 – 2808.

[130] Borkman, M., Storlien, L. H., Pan, D. A., Jenkins, A. B., *et al*., The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. *N. Engl. J. Med.* 1993, *328*, 238 – 244.

[131] Connor, S. L., Connor, W. E., Are fish oils beneficial in the prevention and treatment of coronary artery disease? *Am. J. Clin. Nutr.* 1997, *66*, 1020S – 1031S.

[132] Feskens, E. J., Loeber, J. G., Kromhout, D., Diet and physical activity as determinants of hyperinsulinemia: the Zutphen Elderly Study. *Am. J. Epidemiol.* 1994, *140*, 350 – 360.

[133] Trichopoulou, A., Costacou, T., Bamia, C., Trichopoulos, D., Adherence to a Mediterranean diet and survival in a Greek population. *N. Engl. J. Med.* 2003, *348*, 2599 – 2608.

[134] Givens, D. I., The role of animal nutrition in improving the nutritive value of animal-derived foods in relation to chronic disease. *Proc. Nutr. Soc.* 2005, *64*, 395 – 402.

[135] Serra-Majem, L., Ribas, L., Ngo, J., Ortega, R. M., *et al*., Food, youth and the Mediterranean diet in Spain. Development of KIDMED, Mediterranean Diet Quality Index in children and adolescents. *Public Health Nutr.* 2004, *7*, 931 – 935.

[136] Vincent, S., Gerber, M., Bernard, M. C., Defoort, C., *et al*., The Medi-RIVAGE study (Mediterranean Diet, Cardiovascular Risks and Gene Polymorphisms): rationale, recruitment, design, dietary intervention and baseline characteristics of participants. *Public Health Nutr.* 2004, *7*, 531 – 542.

