Nutrición Hospitalaria

Revisión

N-3 fatty acids in glucose metabolism and insulin sensitivity

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Abstract

Polyunsaturated fatty acids (PUFA) of the n-3 series are essential for normal growth and development. The health effects of these fatty acids include reduction of cardiovascular risk due to antiarrhythmic, antiinflammatory, anti-thrombotic and lipid lowering actions. An increase in unsaturation of the muscle membrane fatty acids is associated with improved insulin sensitivity. Higher proportion of n-3 fatty acids may have beneficial roles, such as antiobesity effects and protection against the metabolic syndrome and type 2 diabetes mellitus through a number of metabolic effects. However, controversy exists on the different effects of n-6 and n-3 polyunsaturated fatty acids as well as on the interacting effect of dietary saturated and monounsaturated fat. In addition, some adverse effects have been described concerning the use of fish oil supplements containing high doses of n-3 fatty acids. Several studies show Eskimos diabetes risk, while results of nutritional interventions on the influence of consuming diets rich in oily fish or other food rich in n-3 fatty acids is very limited. This article reviews the possible mechanisms through which n-3 PUFA are involved in glucose level control and insulin sensitivity. Intervention and epidemiological studies together with recent findings on the nutrigenomic field related with this subject are also briefly reviewed.

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Key words: *N-3 fatty acids. Fish oil. Type 2 diabetes mellitus. Insulin resistance. Nutrigenomics.*

ÁCIDOS GRASOS N-3 EN EL METABOLISMO DE LA GLUCOSA Y LA SENSIBILIDAD A LA INSULINA

Resumen

Los ácidos grasos poliinsaturados (AGP) de la serie n-3 son esenciales para el crecimiento normal y el desarrollo. Los efectos de estos ácidos grasos en la salud incluyen reducción del riesgo cardiovascular debido a acciones antiarrítmicas, antiinflamatorias, antitrombóticas e hipolipemiantes. Un aumento en la insaturación de los ácidos grasos de la membrana muscular se asocia con mejora en la sensibilidad a la insulina. Una mayor proporción de ácidos grasos n-3 puede desempeñar papeles beneficios, tales como efectos antiobesidad y protección frente al síndrome metabólico y la diabetes mellitus tipo 2. Sin embargo, existe controversia sobre los diferentes efectos de los ácidos grasos n-6 y n-3, así como con la interacción entre la grasa saturada y monoinsaturada. Además, se han descrito algunos efectos adversos en relación al uso de suplementos de aceite de pescado con altas dosis de ácidos grasos n-3. Algunos estudios muestran el riesgo de diabetes en esquimales, mientras que los resultados de intervenciones nutricionales sobre la influencia del consumo de pescado graso u otros alimentos ricos en ácidos grasos n-3 son muy limitados. Este artículo revisa los posibles mecanismos a través de los cuales los AGP n-3 intervienen en el control de la glucosa y la sensibilidad a la insulina. Se revisan los estudios epidemiológicos y de intervención junto con los hallazgos más recientes en el campo de la nutrigenómica relacionados con la sensibilidad y resistencia a la insulina.

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Palabras clave: *Ácidos grasos n-3. Aceite de pescado. Diabetes mellitus tipo 2. Resistencia a la insulina. Nutrigenómica.*

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Background

The diet on which humans have evolved contains a ratio of n-6 to n-3 of ~1 whereas nowadays this has been discontinued, which leads to an upset in the functioning of some of our systems.[1] A n-6/n-3 ratio of 4/1 to 6/1 is considered more suitable, although some authors propose a ratio of 1/1.[2] The adoption of westernized lifestyle, characterized by low physical activity and high fat and salt intake, predisposes humans to the development of the metabolic syndrome (MetS), a major public health problem.[3,4] This syndrome includes a series of metabolic disturbances combining insulin resistance, cardiovascular disease and obesity in the same individual.[5,6] Dietary fat contains a variety of saturated, monounsaturated and polyunsaturated triacylglycerols,[2,7] and the role of n-3 fatty acids on glucose levels control is here revised.

Therefore, the aim of the present work is to update existing knowledge on the role of n-3 fatty acids on the glucose metabolism and insulin resistance, and its relationship to the development of some chronic diseases, with special focus on the MetS and type 2 diabetes mellitus (T2DM).

Fatty acids: sources, elongation and desaturation

Fatty acids are the characteristic components of some lipids, composed of a hydrocarbon linear long chain mostly with an even number of carbon atoms and with a carboxylic edge. They are classified into saturated fatty acids (SFA), with simple binding between the carbons, such as palmitic (C16:0) and stearic (C18:0) acids; solids at room temperature; and unsaturated fatty acids, with double bonds between the carbons, which consist of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA).[2] There are three main families of PUFA, namely, n-9, n-6 and n-3 (table I).[8,9] The distinction between n-3, n-6, and n-9 fatty acids is based on the location of the first double bond, counting from the methyl end of the fatty acid molecule. MUFA are represented by oleic acid (C18:1, n-9). Its double bond is between the 9th and 10th carbon atoms, thus it is an n-9 fatty acid. Oils rich in MUFA and PUFA are liquid at room temperature. The fatty acids of the n-3 and n-6 series are also known as essential fatty acids because humans, like all mammals, cannot synthesize them and must obtain them from their diet.[8] Linoleic acid (LA, C18:2, n-6) is the mother fatty acid of the n-6 series while α-linolenic (ALA, C18:3,

Table I
Main fatty acids familis (adapted from 2, 8)

Family	"mother" fatty acid	Source	Structure	Main metabolites
Saturated	Acetic acid (2:0)	Animal fat	No double bounds	(C16:0) Palmitic acid
		Vegetable fat		(C18:0) Stearic acid
Unsaturated fatty acids n-9	Oleic acid (18:1 n-9)	Synthesis from acetate or stearate	First double bound between C_9 and C_{10} respect to the methyl edge	(C20:3 n-9) Eicosatrieonic acid
		Animal fat	H_3C-C-C-C-C-C-C-C = C-R	
		Vegetable oil		
Unsaturated fatty acids n-6	Linoleic acid (18:2 n-6)	Vegetable oil	First double bound between C_6 and C_7 respect to the methyl edge	(C20:3 n-6) dihomo–γ-linolenic acid
		Animal fat	H_3C-C-C-C-C-C = C-R	
Unsaturated fatty acids n-3	α-linolecic acid (18:3 n-3)	Some vegetable oils (perilla, flaxseed, canola, soybean, rapeseed), walnuts	First double bound between C_3 and C_4 respect to the methyl edge	(C20:5 n-3) eicosanpentaenoic acid
				(C22:6 n-3) docosahexaenoic acid
			H_3C-C-C = C-R	
		Fish and fish oils		
		Other sources: lean meat and meat products, offal, egg yolk, milk and dairy products		

Table II
Essential fatty acid metabolic desaturation and elongation of n-3 and n-6 PUFA

Linoleate serie	Linolenate serie
C18:2 n-6 linoleic acid	C18:3 n-3 Alpha-linoleic acid
↓ Δ⁶ desaturase	↓ Δ⁶ desaturase
C18:3 n-6 Gamma-linolenic acid	C18:4 n-3
↓	↓
C20:3 n-6 dihomo-gamma-linolenic acid	C20:4 n-3
↓ Δ⁶ desaturase	↓ Δ⁵ desaturase
C20:4 n-6 Arachidonic acid	C20:5 n-3 Eicosapentaenoic acid
↓ Elongation	↓ Elongation
C22:4 n-6 Docosatetraenoic acid	C22:5 n-3 Docosapentaenoic acid
↓ Elongation	↓ Elongation
C24:4 n-6 Tetracosatetraenoic acid	C24:5 n-3 Tetracosapentaenoic acid
↓ Δ⁴ desaturase	↓ Δ⁴ desaturase
C24:5 n-6 Tetracosapentaenoic acid	C24:6 n-3 Tetracosahexaenoic acid
↓ Partial β-oxidation	↓ Partial β-oxidation
C22:5 n-6 Docosapentaenoic acid	C22:6 n-3 Docosahexaenoic acid

n-3) of n-3 series. Docosahexaenoic (DHA, C22:6, n-3), eicosapentaenoic (EPA, C20:5, n-3), and arachidonic (AA, C20:4, n-6) acids are PUFA playing very important physiological roles.

The n-3 and n-6 families of PUFA are important components of practically all cell membranes.[1] Whereas cellular proteins are genetically determined, the PUFA composition of cell membranes is to a great extent dependent on dietary intake.[8]

Although LA and ALA are the really essential fatty acids in an adult's diet, long chain PUFA (LCPUFA), DHA and AA, are also essential for the foetus' complete development as foetal desaturases and elongases are partially inefficient.[3] Therefore, foetus essential fatty acids and LCPUFA have to come from the mother, who obtains them mostly from her diet.[3]

Table II shows the most accepted desaturation-elongation pathway for n-3 and n-6 PUFA. The existence of Δ⁴ desaturase has been discussed and the transformation of C22:4 n-6 or C22:5 n-3, into C22:5 n-6 and C22:6 n-3 respectively, has been attributed to a combination of the elongase-descarboxilase system.[2,3]

Dietary sources of ALA are mainly green leafy vegetables, linseed (also called flaxseed), soybean, canola, rapeseed oils, walnuts and Brazil nuts. N-3 LCPUFA, especially EPA and DHA, are found in oily fish, either from marine or farm origin, such as salmon, sardines, mackerel and tuna. N-6 PUFA occurs mostly in the diet as LA and can be found in sunflower oil, corn, rapeseed, safflower, oils, and many nuts, grains and seeds (table I).[1,8,10]

Insulin resistance and the metabolic syndrome

Insulin resistance is usually defined on a metabolic level as inefficient insulin function in skeletal muscle, liver and adipocytes. This hampers the normal role of insulin whereby it causes increased muscle cellular glucose uptake, glycogen synthesis, and cessation of hepatic glucose production.[11,12] Table III summarizes the methods and indexes used to assess insulin resistance.

Insulin resistance is a growing worldwide phenomenon, which has progressively developed over years, and finally, if unchecked, predisposes to cardiovascular disease and T2DM.[13,14] In current present society this is the consequence in lifestyle changes during the infant and adolescent stages: a decrease in physical activity while increasing body weight and nutrition changes (quick spread and success of fast food instead of the traditional and home-made cooking). Those changes have led to a loss of blood glucose control that might result from failure of the beta cells to secrete insulin, resistance of the tissues to its action, or a combination of both. This is because under a high-fat and high-sucrose diet insulin secretion increases to accommodate the need to store glucose and excess fatty acids.[14-20] The condition of insulin resistance is tightly coupled with obesity and cardiovascular pathology; these conditions are collectively called the MetS'or decades ago 'syndrome X".[11,20,21] Recently it is also called Cardiometabolic Symdrome.

MetS is generally used to indicate a clinical situation in which different degrees of hypertension, impaired glucose tolerance, atherogenic dyslipidemia, central fat accumulation, as well as prothrombotic and proinflammatory states cluster together in the same individual.[22,23] Table IV reflects the different diagnosis criteria for MetS. Such a concurrence of disorders increases the probability of suffering from cardiovascular disease or T2DM, possibly more than what the sum of the single risk factors would predict. In 1998 the WHO[24] published consensus definitions of MetS, based on serum values of triacylglycerols, HDL-cholesterol, blood pressure, and measurements of central obesity and fasting glucose. Later definitions increase accuracy of central obesity and insulin resistance,[25] as presented in table IV.

During the last decade, the MetS has progressively become a major public health problem,[26] both in healthy societies and in developing countries. Now, it is approaching epidemic proportions worldwide and its spreading prevalence is strictly associated with the adoption of a 'westernized'lifestyle, which is characterized by lack of physical activity, excessive food intake, a combination of factors leading to overweight and obesity. In fact, obesity, and particularly visceral obesity, seems to be a major determinant of insulin resistance, hence preparing the path to the clustering of metabolic and non-metabolic factors embraced under the descriptive term of MetS.

Test	Labour intensity	Method	Reproducibility	Uses	Interpretation of results
Euglycaemic clamp[130]	Blood glucose measurements every 3 min in addition to plasma samples over 150-180 min	Constant rate i.v. insulin infusion and variable rate i.v. glucose infusion	Between subject CV 21%	Useful research tool for studying physiology; often regarded as the "gold standard"	Insulin resistance is estimated from the ratio of the mean glucose infusion to the mean insulin concentration over the last 20-30 min of the clamp
Short ITT[131,132]	Nine samples over 15 min	i.v. bolus of insulin (0.1-0.5 U/kg)	Between subject CV 26%. Within subject CV 6-13%	To study physiology: short duration of test is an advantage	Insulin sensitivity is estimated from the slope of the regression line of the logarithm of glucose concentrations against time
HOMA and QUICKI[133-136]	Three basal samples at 5 min intervals	Overnight fast	CV 10%	Useful in epidemiological studies or longitudinally within and individual	Read off computer-derived nomogram or computerized table. HOMA and QUICKI are highly correlated
CIGMA[137]	Three samples at 50, 55 and 60 min	i.v. glucose infusion (5 mg/kg per min) for 60 min	CV 21%	Useful in epidemiological studies or longitudinally within and individual	Read of computer-derived nomogram or computerized table
FSIVGTT— minimal model[138]	Twenty-five samples over 3 h	i.v. glucose bolus ± i.v. bolus of tolbutamide or insulin at 20 min	Within subject CV 20%	Useful research tool for studying physiology	Analysis of results requires a computer program
IST[139]	Six samples at 0, 60, 120, 150, 160, 170, 180 min	Constant rate infusion of glucose (240 mg/m^2 per min), insulin (25 mU/m^2 per min) and somatostatin (350 µg/h) for 150-180 min	Within subject CV 10%	Useful research tool for studying physiology. Time-consuming but less labour-intensive than FSIVGTT or clamp	SSPG is calculated from the mean of the glucose levels over the last 30 min
GITT[140]	Four samples at 0, 5, 11 and 20 min	i.v. glucagon at baseline and i.v. insulin at 30 min	CV 13%	Relatively cheap Measures insulin sensitivity and β-cell function	A first-order rate constant for glucose disappearance was estimated from the slope of regression line in plasma glucose

ITT: Insulin Tolerance Test. HOMA: Homeostatic Model Assessment. QUICKI: Quantitative Insulin Sensitivity Check Index. CIGMA: Continuous Infusion of Glucose with Model Assessment. FSIVGTT: Frequently Sampled Intravenous Glucose Tolerance Test. IST: Insulin Suppression Test. SSPG: Steady State Plasma Glucose. i.v.: intravenous.

The ability of body tissue to react to insulin becomes progressively more compromised as it moves maybe for ten to twenty years, through the stages of insulin resistance. During this stage of impaired insulin action, insulin secretion also becomes higher in an effort to correct the condition, until the beta cells of the pancreas are depleted and cease production, resulting in full-blown T2DM.[27]

Having reached this point, the already-known healthy properties of the n-3 fatty acids and their potential ones are of great interest to us.

N-3 fatty acids health effects

On September 8, 2004, the U.S. Food and Drug Administration gave «qualified health claim» status to EPA and DHA n-3 fatty acids, stating that «supportive but not conclusive research shows that consumption of EPA and DHA ω−3 fatty acids may reduce the risk of coronary heart disease» [27]

N-3 fatty acids also exert some different good physiological actions, however several effects remain unclear, as is reflected in table V. The n-3 PUFA effects on glucose metabolism and its disorders will be reviewed thereafter.

Results from epidemiological and dietary intervention studies have shown that n-3 PUFA represents a class of powerfully bioactive compounds and that an adequate dietary intake of n-3 PUFA plays a critical role in human health in relation to non-communicable diseases.[4]

The effects of n-3 PUFA on plasma lipid levels have been documented in numerous studies.[28] The lowering-

Table IV
Current definitions of metabolic syndrome[24,25,141]

NCEP ATP III (≥ 3 criteria)	AHA/NHLBI (≥ 3 criteria)	IDF (Obesity + ≥ 2 other criteria)
Waist circumference		
> 102 cm (men)	102 cm (men)	Ethnicity specific
88 cm (women)	88 cm (women)	94-90 cm (men)
		88 cm (women)
Triglycerides		
≥ 150 mg/dl	≥ 150 mg/dl	≥ 150 mg/dl
	or treatment for	or treatment for
	hypertriglyceridemia	hypertriglyceridemia
High-density lipoprotein cholesterol (HDL-C)		
< 40 mg/dl (men)	< 40 mg/dl (men)	< 40 mg&dl (men)
< 50 mg/dl (women)	< 50 mg/dl (women)	< 50 mg/dl (women)
	or treatment for	or treatment for
	low HDL-C	low HDL-C
Blood pressure		
≥ 130/85 mmHg	≥ 130/85 mmHg	≥ 130/85 mmHg
or treatment	or treatment	or treatment
for hypertension	for hypertension	for hypertension
Fasting glucose		
100-125 mg/dl	≥ 100 mg/dl	≥ 100 mg/dl
	or treatment	or diagnosis
	for hyperglycemia	or diabetes mellitus

Table V
Health effects of eicosapentanoeic acid (EPA) and docosahexanoeic acid (DHA)

Beneficial effects

- Disminishes most of the CVD risk factors, such as abnormal levels of blood pressure, serum triacylglycerols, Lp (a), fibrinogen, decrease platelet aggregation, blood viscosity, and inflammation; and increases erythrocyte deformability, thus decreasing the tendency of thrombus formation[1,4,7,34,37,142,143].
- Anti-inflammatory activities. Improve joint pain in patients with rheumatoid arthritis, as demonstrated in clinical trials using EPA and DHA in the form of fish oil along with antirheumatic drugs, have a beneficial effect in patients with ulcerative colitis, and improve the skin lesions in psoriasis patients[4,8,47,79,144-146].
- They are essential in growth and development [8].
- They are essential for normal brain development and visual function[8,47,147].
- May be beneficial in attention-deficit/hyperactivity disorder, schizophrenia and depression[4].
- Delays tumour appearance and decreases both the rate of growth and the size and number of tumours[4,8,147].
- Anti-arrhythmic [8].

Adverse effects

- High consumption of oily fish involves increased intake of toxic elements such a mercury, arsenic and lead[68,148]. In addition, mercury is associated to hypertension[98,99].
- Increased bleeding time and clinical bleeding (nasal, hematuria, gastro-intestinal, and other bleeding). This has been observed in populations with CVD undergoing pharmacological treatment[50].

cholesterol effect in patients with established high cholesterol plasma levels has been reported although this effect is lower in healthy subjects which means it is health-status sensitive.[29] The hypotriglyceridemic effect of n-3 fatty acids from diets rich in oily fish or fish oil supplements is well documented.[30-33] Concerning the influence on serum cholesterol, the majority of studies found decreases in total cholesterol[34-36] and increases in HDL-cholesterol[32,37-39] though the effect on LDL-cholesterol is controversial.[40] Several reports indicate that n-3 fatty acids do not change LDL-cholesterol,[41] others show an increase after consumption of high doses of n-3.[32,38,39,42] A higher susceptibility of LDL particles to oxidation has been observed[38,43] but not always[44] in individuals given n-3 PUFA. This discrepancy should be related to the experimental conditions, fish intake *vs* n-3 supplement and doses used. Griffin et al[39] compared 4 diets with an n-6/n-3 ratio between 5:1 and 3:1. The diets were enriched with ALA, EPA and DHA, or both. It was found that n3-PUFA (n-6/n-3 ratio of 3) decreased small dense-LDLs and postprandial triacylglycerols plasma levels, HDL-cholesterol did not change but HDL_2 increased. Our group found that a dose of 0.8 g/day of fish oil induces the production of potentially less oxidized LDL in young women.[45]

In addition, the effects of n-3 fatty acids on serum lipids depend on the subject and whether the amount of SFA and MUFA in the diet are kept constant.[1,46] A total of 1.5 to 3.5 g of n-3 PUFA intake per day is required to achieve a significant effect in hyperlipaemic patients (with variable intakes of SFA and MUFA). Reductions of the triacylglycerol levels of 25-40% can then be obtained.[47,48]

Södergren et al [49] compared in a randomized crossover study, the influence of a rapeseed oil-based diet (rich in ALA and oleic acid) and a SFA-rich diet on lipid levels and peroxidation in a group of moderately hyperlipidemic subjects. Total cholesterol, LDL-cholesterol and the LDL-cholesterol/HDL-cholesterol ratio were lower after the rapeseed oil diet, while HDL-cholesterol and total triacylglycerol levels were similar after the two diets and there were no changes in the degree of lipid peroxidation. The authors attributed these results to the content of antioxidants in the rapeseed oil-diet and to other undetermined carry-over effects.

However, side effects, such as the association of oily fish consumption with mercury, lead and arsenic toxicity have also been reported. At present, fish traceability and safety are of great concern, but considering the risk/benefit ratio of fish consumption, the benefits are higher than the risks so public health recommendations include regular intake of these marine sources.[50]

The World Health Organization[51] recommends population nutrient intake goals for n-3 PUFA to represent 1-2% of total energy intake,[52,53] after consensus on its diet effects on chronic diseases. In agreement with this, Spanish guidelines establish a 0.5-1% of total energy to be ALA and 0.2-0.5% to be EPA plus DHA, within a n6/n3 ratio from 4 to 10.[2,54]

Effects of n-3 PUFA intake on insulin sensitivity

Fat level and type of fat

Few studies have addressed whether the fatty acid composition of the diet influences the fatty acid profile of skeletal muscle phospholipids and triacylglycerols. The fatty acid composition of the cell membrane is a dynamic system, and the regulation mechanisms are not fully understood. Both genetic[55] and lifestyle-related factors, including diet[56,57] and physical activity,[58] seem to play a part in determining the fatty acid composition of skeletal muscle phospholipids. Table VI summarizes different studies which have observed n-3 PUFA and MUFA influence on insulin resistance, insulin sensitivity, and fasting plasma glucose levels in different type of populations.

Andersson et al[59] investigated whether there is a correspondence between dietary fatty acid composition and fatty acid profile of skeletal muscle and triacylglycerols. They randomly assigned diets containing a high proportion of either SFA or MUFA (total fat, 36% of energy) to two groups of healthy men and women; one group started with a higher proportion of SFA than that of MUFA in the diet, and the other with the opposite (higher MUFA proportion in diet) for 3 months. Within each diet group, there was a second random assignment to supplementation with fish oil capsules containing 3.6 g n-3 PUFA/day (EPA and DHA) or placebo. A skeletal muscle biopsy sample was taken after the diet period, revealing that the fatty acid composition of skeletal muscle lipids reflects the fatty acid composition of the diet in healthy men and women.

This is important because skeletal muscle is an important tissue for the whole-body energy metabolism, including insulin-stimulated glucose uptake[60] and fatty acid oxidation.[61] The fatty acid composition of skeletal muscle phospholipids has been related to peripheral insulin sensitivity[18,62-64] and obesity[63,65] in several human populations. Figure 1 shows PUFA
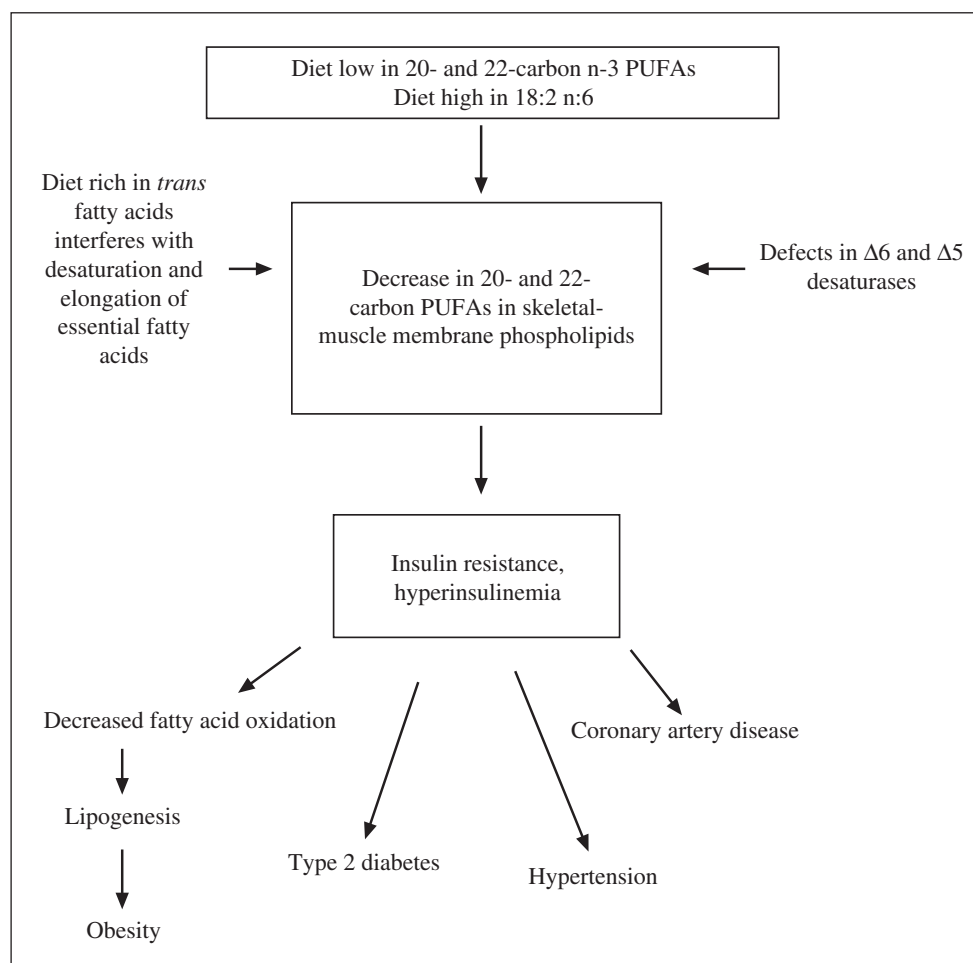

Fig. 1.—Hypothetical scheme of the effects of dietary 20- and 22-carbon PUFA on the composition of the PUFAs in skeletal-muscle membrane phospholipids, and their relations with insulin resistance, hyperinsulinaemia, and chronic disease (modified from 127).

Table VI
PUFA and MUFA effects on glucose metabolism

Author	Subjects	Observation	Intervention/measurements	Conclusion
Andersson et al.[59]	Healthy men and women	Correspondence between dietary fatty acid composition and fatty acid profile of skeletal muscle and triacylglycerols	2 groups: SFA diet and MUFA diet for 3 months, randomly assigned. Within each group, second random assignment to supplementation with fish oil capsules containing 3.6 g EPA + DHA/day or placebo	Fatty acid composition of skeletal muscle phospholipids reflects the fatty acid composition of the diet
Borkman et al.[18]	Patients undergoing coronary artery surgery/healthy men	Relationship between fatty acid composition of skeletal muscle phospholipids and insulin sensitivity	Muscle biopsy and assessment of fasting serum insulin levels (in surgery patients) or euglycemic-clamp studies (in healthy subjects)	Decreased insulin sensitivity is associated with decreased concentrations of PUFA in skeletal-muscle phospholipids
Pan et al.[63]	Adult male Pima Indians (usual diet very low in n-3 PUFA)	Correspondence between skeletal muscle lipid composition and insulin resistance	Euglycaemic clamp, muscle biopsy	Low levels of n-3 PUFA in skeletal muscle lipid membranes
Baur et al.[56]	Young children	Correspondence between insulin sensitivity and fatty acid composition of serum lipids and skeletal muscle phospholipids	Skeletal muscle biopsies and fasting blood samples	Higher levels of LCPUFA in the phospholipid of skeletal muscle is associated with lower fasting plasma glucose. Early changes in skeletal muscle membrane phospholipid saturation may play a role in the subsequent development of diseases associated with insulin resistance
Vessby et al.[64]	70 year old men	Correspondence between insulin sensitivity and fatty acid composition of serum lipids and skeletal muscle phospholipids	Health survey. Insulin sensitivity measured by the euglycaemic hyperinsulinaemic clamp technique. The fatty acid composition of the serum cholesterol esters was determined	Fatty acid composition in serum and of the phospholipids of skeletal muscle may influence insulin action in elderly men
Browning et al.[77]	Overweight and obese premenopausal non-diabetic women	Correspondence between n-3 PUFA supplementation and lower insulin sensitivity	Weight-reducing diet plus n-3 PUFA supplementation (1.3 g EPA plus 2.9 g DHA) during 12 weeks	Improvement in insulin sensitivity after the n-3 PUFA supplementation
Mori et al.[48]	Overweight hypertensive subjects	Effects of an increase of n-3 PUFA in a weight loss diet and serum glucose and insulin metbolism	Randomly assignment of either a daily fish meal (3.65 g n-3 PUFA), a weight-loss diet, the 2 treatments combined, or a control group for 16 weeks	Improvement in glucose-insulin metabolism by oily fish plus the weight-loss diet, but no influence of oily fish treatment alone
KANWU study, Vessby et al.[46]	Healthy men and women	MUFA effects on insulin sensitivity/secretion	2 groups: SFA diet and MUFA diet for 3 months, randomly assigned. Within each group, second random assignment to supplementation with fish oil capsules containing 3.6 g EPA plus DHA/day or placebo	Decreasing SFA and increasing MUFA improves insulin sensitivity but has no effect on insulin secretion
Pérez Jiménez et al.[80]	Healthy men and women	PUFA effects modulated by MUFA	3 different diets during 4 weeks. Diets provided consisted of SAT diet (38% of total fat and 12% of MUFA), reduced fat diet (28% of total fat and 12% of MUFA) and Mediterranean diet (38% of total fat and 22% of MUFA)	MUFA improves insulin sensitivity
OPTILIP study, Griffin et al.[39]	45-70 aged men and women	Influence of amount and quality of dietary fat on insulin resistance	4 diets providing 6% of energy as PUFA with a n-6/n-3 ratio between 3-5, control diet had a n-6/n-3 ratio of 10. The diets were enriched in EPA, DHA, or both. Insulin sensitivity and resistance was assessed with the HOMA and QUICKI models	No modification in insulin sensitivity/resistance

influence on skeletal-muscle membrane phospholipids. An increase in 20- and 22-carbon PUFA, ie, AA, EPA, and DHA, leads to increases in membrane fluidity, number of insulin receptors, and insulin action.[66,67]

Early work[68] suggested a population-wide relationship between dietary fat (amount and quality) and obesity, diabetes mellitus and other degenerative diseases in which there is an increased affluence.

Epidemiological data suggest that subjects with higher intakes of fat are more prone to develop disturbances in glucose metabolism than subjects with lower intake of fat. Animal studies have given evidence that high-fat diets affect glucose metabolism negatively, but in humans (mostly T2DM patients) the results have been inconsistent and are likely confounded with differences in body weight.[67] Greenland Inuits, who have a high total fat intake rich in PUFA (both n-3 and n-6), have surprisingly low prevalences of T2DM and of other glucose metabolism disorders.[68,69] In studies in which the fatty acid composition of a high-fat diet has been modified to contain a higher proportion of unsaturated fat, an improvement of glucose metabolism, compared to a high-SFA diet, has been observed. Other studies[46,70] using isocaloric diets with 44% of energy as total fat, confirm that the diet rich in SFA (PUFA/SFA ratio = 0.2) resulted in less favourable glucose tolerance, especially in subjects with type 4 hyperlipidemia (elevated triglyceride levels carried by VLDL). In normal subjects insulin concentrations, during the oral glucose tolerance test, were inversely associated with the dietary intake of PUFA and positively associated with the intake of SFA.[71]

Therefore, it seems that there are confounding factors in the human results. Among these, the overweight/obese condition mentioned above, drug therapy, and the dietary n-6 and n-3 imbalance should be highlighted.

The hypothesis that a high background intake of n-6 PUFA could diminish beneficial effects of n-3 LCPUFA supplementation on insulin sensitivity was tested in Asian Indians living in the United Kingdom who used to have a significantly higher intake of PUFA, mainly as n-6 PUFA from vegetable oils, and a lower intake of n-3 LCPUFA than in whites.[72] Volunteers received either a moderate or a high n-6 PUFA diet during a 6-week period, after which both groups were supplemented with 4.0 g fish oil/d (EPA+DHA) for an additional 6 weeks in combination with the dietary treatment. An insulin sensitivity test was performed after each of the 6 week dietary intervention periods; the result was that the background dietary n-6 PUFA concentration did not modulate the fish-oil supplementation measures of insulin sensitivity in this ethnic group. This finding should be explained considering that a 6 week-experimental period is enough to change membrane phospholipids, which is in agreement with most of the experimental periods used in lipid clinical assays.

Borkman et al[18] determined the relationship between the fatty-acid composition of skeletal-muscle phospholipids and insulin sensitivity in patients undergoing coronary artery surgery in normal men. In both groups, skeletal muscle samples were obtained. They observed (determining fasting serum insulin levels and using the euglycaemic-clamp technique) that the more PUFA percentage of composition in the phospholipid fraction, the less insulin resistance, thus raising the possibility that changes in the fatty-acid composition of muscle modulate the action of insulin.[51,73] Similarly, other authors[63] in a study with adult male Pima Indians (a population with the highest reported incidence of T2DM in the world[74]) observed significant relationships between skeletal muscle membrane phospholipid fatty acid composition and both insulin action and adiposity, by determining insulin action (euglycaemic clamp method), percentage body fat, and muscle phospholipid fatty acid composition. Results revealed that insulin action was diminished as membrane unsaturation was low, and that insulin action is positively correlated with the percentage of C-20 and C-22 PUFA, including n-3 fatty acids.[75,76] As indicated above, the fatty-acid composition of skeletal-muscle phospholipids reflects the profile of the fat ingested.

Improvement in insulin sensitivity after 12 weeks of n-3 fatty acids supplementation (1.3 g EPA and 2.9 g DHA) has been also observed in overweight and obese (BMI 24-44 kg/m^2) premenopausal non-diabetic women.[77] The combination of oily fish consumption with a weight-reducing diet decreased more body weight than only the dietary regimen.[48] In addition, n-3 fatty acids supplementation improved glucose and insulin metabolism in overweight patients treated for hypertension.[39,48]

In contrast, approximately 1 g/day of EPA plus DHA, using a food-based intervention, was unable to modify insulin sensitivity or postprandial lipase activity in men and women aged 45-70.[39]

In children younger than the age of 2, a significant inverse correlation between a fasting plasma glucose concentration and the percentage of LCPUFA in skeletal muscle membrane phospholipids has also been reported.[56]

The ingestion of both n-6 and n-3 fatty acids has been found to suppress hepatic lipogenesis, reduce the hepatic output of triglycerides, enhance ketogenesis, and induce fatty acid oxidation in both the liver and the skeletal muscle. Taken together, these effects might explain an actual improvement in glucose uptake and insulin sensitivity after n-3 (but also n-6) fatty acid ingestion. Insulin sensitivity may improve as a result of the effects of fatty acid intake on membrane fluidity. The improvement in glucose uptake after membrane enrichment with PUFA is apparently related to an increase in the residency time of glucose transporters type 1 and 4 (GLUT1 and GLUT4) in the plasma membrane (which leads to an expansion of the intracellular pool of glucose-6-phosphate and to increased skeletal muscle glycogen synthesis).[78]

There is controversy concerning the effects of MUFA on insulin sensitivity. In fact, dietary protocols

have often ignored the influence of MUFA modulating SFA, PUFA or specifically n-3 fatty acids actions. After comparing a group of healthy moderately hyper-lipidemic subjects on a rapeseed oil (rich in ALA and oleic acid) diet, who were provided with 9 g/day of total n-3 fatty acids, with a SFA-rich diet during two consecutive 4 week periods separated by a 4 week wash-out period, lower fasting plasma glucose was observed but there were no changes in insulin and free fatty acids.[49] This effect should be attributed not only to the relatively high dose of n-3 fatty acids in this study but also to the ~50% higher MUFA that the rapeseed oil provided. Nevertheless, in animal models, high n-3 fat intake favours glucose metabolism.[79]

Reinforcing the idea that MUFA improves insulin sensitivity, it was also observed that following consumption of three different diets for a 4 week-period in healthy men and women insulin sensitivity was clearly improved with a Mediterranean type diet, which is a relatively high fat diet rich in MUFA. Diets provided consisted of a SAT diet (38% total fat of which 12% MUFA), reduced fat diet (28% total fat of which 12% MUFA) and Mediterranean diet (38% total fat of which 22% MUFA).[80]

The KANWU study[46] took also into account the MUFA interacting effect. Healthy adults randomly chosen were given a controlled isoenergetic diet containing either a high proportion of SFA or MUFA diet during 3 months. Within each group, there was a second assignment at random with a supplement of fish oil (3.6 g n-3 fatty acids/d) or placebo. Insulin sensitivity was significantly impaired on the SFA diet but did not change on the MUFA diet. Insulin secretion was not affected and the addition of n-3 fatty acids diet influenced neither insulin sensitivity nor insulin secretion. The favorable effects of substituting a MUFA diet by a saturated fatty acid diet on insulin sensitivity were only detected at a total fat intake below median, but were not noted in individuals with a high fat intake (> 37E%).

Our research group recently studied the effects of consuming five portions of oily fish or red meat during 2 months on insulin resistance in young women. The oily fish diet induced an increase in n-3 intake reaching 3 g/day, without changes in SAT fat and a slight reduction in MUFA intake (that was around 50% total fat). Under these experimental conditions the oily fish diet induced a significant decrease in insulin levels and insulin resistance (determined by HOMA and QUICKI).[81]

Clinical studies in diabetic subjects

Early studies, such as the one performed by Jensen et al[82] with type 1 diabetes patients receiving cod-liver oil (natural source rich in n-3 PUFA), showed improvement in complications attributable to microvascular disease but blood glucose concentrations were unchanged.

Some studies have been conducted on the effects of n-3 PUFA in patients with T2DM.[83] In most of them, fish-oil consumption rose plasma glucose concentrations. In many of these studies, however, the number of subjects was small, the dose of n-3 fatty acids was > 3 g/day and controls were insufficient.

In a randomized, double-blind, placebo-controlled, crossover trial, patients with T2DM consumed 6 g/day of n-3 fatty acids (EPA plus DHA) for 6 months in addition to their usual oral therapy.[84] Fasting serum glucose concentrations increased 11% during the n-3 PUFA phase and 8% during the placebo phase (olive oil). Thus a non-significant net glucose increase of 3% was observed. This study showed convincingly that n-3 PUFA intake, along with oral therapy for diabetes, has no adverse effects on glycaemic control.

The review by De Caterina et al[19] concluded that the administration of n-3 fatty acids does not apparently affect glucose control in patients with type 1 diabetes but it is notable that the studies they reviewed included a relatively low number of patients and were of short duration.

Another review article[85] based on very few observations stated that in patients with T2DM n-3 fatty acids had an adverse effect on metabolic control of diabetes, but more recent studies report no adverse effect of dietary n-3 fatty acids on glucose control. The study by Sirtori et al[86] is of special interest because of the relatively high number of patients treated, and its duration (1 year). Eighty nine patients with T2DM were randomized to EPA plus DHA 2.6 g/day for the first 2 months and then 1.7 g/day for the next 4 months. Olive oil control was used as placebo. Treatment was then continued in a non-blind manner up to 1 year. No significant differences were observed between the treatment groups in relation to fasting glucose or insulin levels. Supplementation with 1.7 g/day to all patients for a further 6 months produced no deterioration of glucose control after 1 year of treatment. In this study, there were, however, patients in whom metabolic control deteriorated during treatment with n-3 fatty acids, but whether this was caused by n-3 fatty acids or was part of the natural history of the disease is uncertain.

Delarue et al[87] observed that in patients with T2DM, fish oil dietary supplementation fails to reverse insulin resistance for unclear reasons and conversely, in healthy humans, fish oil has many physiological effects, indeed, it reduces insulin response to oral glucose without altering the glycaemic response. Later Mostad et al[88] studied the effects of fish oil supplements on metabolic variables in subjects with T2DM by studying short-term (1 week) and longer-term (9 weeks) effects of n-3 fatty acids. They provided T2DM non-hypertriacylglycerolemic subjects 1.8 g EPA, 3.0 g DHA, and 5.9 g total n-3 PUFA in the form of fish oil. The control group received a similar amount of corn oil/day (8.5 g LA). Glucose concentrations (home-monitorized) were ≈ 1 mmol/L higher in the fish oil group than in the corn oil group at the end of the inter-

vention. Glucose utilization measured by using an isoglycemic clamp technique was lowered in the fish oil group compared with that in the corn oil group at the end of the intervention, concluding that a high intake of fish oil moderately increases blood glucose and decreases insulin sensitivity in T2DM subjects in a time-dependent manner.

Historically, epidemiologic studies have reported a lower prevalence of impaired glucose tolerance and T2DM populations consuming large amounts of the n-3 LCPUFA found mainly in fish.[89]

Several clinical studies in the 1980s and early 1990s reported adverse effects on blood glucose control and insulin activity in subjects with T2DM who consumed large amounts of fish.[86,90,91] It is now believed that these deleterious effects were largely attributable to the high doses used (e.g. 10 g/day fish oil or more). Recent studies using low doses of n-3 LC-PUFA, ranging from 1 to 2 g/day, have reported no deterioration of glucose control.[92-94] In addition, one study shows that the increase in glycated hemoglobin associated with daily fish consumption providing 3.6 g/day n-3 PUFA could be prevented by moderate exercise (55% to 65% maximum oxygen consumption/min).[95,96]

Epidemiological studies

Stene et al[97] performed an epidemiological study in Norwegian pregnant women. They discovered that the use of cod liver oil during pregnancy was associated with lower risk of Type I diabetes in the offspring. This effect was found only in mothers taking cod liver oil, not in mothers taking multivitamin supplements.

Nevertheless, pregnant women should be wary and not consume large amounts, since researchers in Iceland report that a high intake of cod liver oil is associated with a nearly five-fold increased risk of gestational hypertension[98], although this study did not control for mercury, which can be present in harmful amounts in fish, and which is another cause of hypertension and cardiovascular disease.[99,100]

T2DM was less prevalent among Japanese islanders compared to their mainland counterparts.[100] Lower prevalence was attributed mainly to diets rich in n-3 LC-PUFA. The incidence of T2DM has increased rapidly among native and migrant populations, as much as 80% among Alaskan natives.[101] The increase has been associated with the greater consumption of non-indigenous foods, changes in lifestyle, and fatty acid imbalance.[102,103]

In the Finnish and Dutch cohorts of the Seven Countries Study, fish consumption was inversely related to 2-hour glucose levels during a 20-year follow-up of male participants.[71] Fish consumption was associated with reduced risk of developing impaired glucose tolerance.[104]

In Iceland, despite the high prevalence of overweight people and obesity, the prevalence of T2DM is lower than in other Nordic countries. Thorsdottir et al[105] reported that the prevalence of T2DM in Icelandic men was inversely associated with both the n-3 PUFA and EPA content of milk, and positively associated with the ratio of n-6/n-3 fatty acids in milk. Icelandic milk contains significantly more n-3 LC-PUFA than milk in other Nordic countries ($0.22 \pm 0.05\%$ *vs* $0.06 \pm 0.01\%$ for Norway and $0.03 \pm 0.01\%$ for Denmark), mainly because animal fodder contains fish meal. Icelandic milk is also lower in n-6 PUFA. Daily consumption of 500 mL Icelandic milk for a week provides about the same amount of EPA as one 100-g serving of sea trout (176 *vs* 165 mg EPA). In this population, the unique composition of dairy fat could be protective against T2DM.

By contrast, in a study of nearly 36,000 older Iowa women who did not have T2DM at enrolment, diabetes incidence after 11 years was positively associated with n-3 LC-PUFA consumption.[74,106] After adjusting for other dietary fat, only vegetable fat was related to diabetes risk and appeared protective.

Van Dam et al[107] reported in the US Health Professional Follow-Up Study that n-3 LC-PUFA intake was not associated with the risk of diabetes in more than 42,000 men, but total and saturated fat intake increased risk. Promising preliminary evidence suggests that the development of T2DM can be retarded or prevented by increased consumption of n-3 LC-PUFA and reduced consumption of SFA as was reported by Ebbeson.[103,108]

Nutrigenomic aspects

The sequential steps preceding T2DM: failure of insulin action at the cells, compensatory increase in insulin secretion, hyperglycaemia, decreased β-cell function, and the concomitant cascade of metabolic alterations, are all the result of polygenic and environmental factors. In addition, since cardiovascular disease and obesity are also multigenic and multifactorial pathologies, we are far from understanding the genetic-diet interaction behind the MetS, particularly in relation to the interacting influence of the different PUFA and in particular of n3 fatty acid intakes. Nevertheless, here we briefly present the genetic variants that have been associated with insulin resistance and the possible link with fat intake.

Genes related to transport of insulin have been investigated. A case in point is the insulin receptor substrate (IRS) that plays a critical role in hepatic insulin signalling and expression of genes involved in gluconeogenesis, glycogen synthesis and lipid metabolism,[109] and the insulin-responsive GLUT4, that plays a key role in glucose uptake and metabolism in insulin target tissues.[110] However, no conclusive results are available concerning specific polymorphisms of these genes and higher risk of insulin resistance, T2DM or the MetS.

Other candidate genes are adiponectin and the adiponectin receptors. Adiponectin is an adipokine secreted by adipocytes that enhances insulin sensitivity and has anti-inflammatory action. In skeletal muscle, it induces glu-

cose uptake and energy expenditure, while in liver it reduces glucose uptake and fat accumulation, thus it protects against MetS. There are many reports concerning the metabolic effects of variation in the gene coding for adiponectin, named *ADIPOQ*. Meta-analyses of these studies support the hypothesis that variability in this gene contributes to the modulation of circulating adiponectin levels and the risk of insulin resistance and CVD. Two linkage disequilibrium blocks have been identified: in the 5'block, the g.-11391G → A variant has modest but significant influence on adiponectinaemia, in the 3'block, the g.+276G → T variant is a strong determinant of insulin resistance and CVD though only marginally significant for adiponectin levels. In contrast, results in relation to genetic variants in the adiponectin receptor gene are still very limited and inconclusive.[111]

Interestingly, serum levels of adiponectin can be modulated by peroxisome proliferator-activated receptor (PPAR)-γ agonists. Polymorphisms in PPARs isoforms may be among the most important single-gene contributors to dyslipidemias, insulin resistance, and T2DM.[112]

PPARs are central regulators of lipoprotein metabolism and glucose homeostasis that are considered particularly useful for improving glycaemic control and comorbidities in patients with T2DM.[113] There are three types: PPAR-α, PPAR β/δ, and PPAR-γ. Clinical trials of PPAR-α[114] agonists have demonstrated efficacy in reducing cardiovascular events; however, these benefits have been confined to subgroups of patients with low levels of HDL-cholesterol or high levels of triacylglycerols, so future studies of PPAR-γ agonists or dual PPAR-α/γ agonists require further delineation of the risk profile to avoid adverse outcomes in susceptible patients. PPAR-γ are nuclear receptors that positively modulate insulin sensitivity.[115]

The finding that PPAR-γ are molecular targets of thiazolidinediones (TZDs), orally active insulin-sensitizing agents increasingly used for treatment of T2DM, represents a new step in knowledge of insulin resistance linked to cardiovascular diseases.[116-118] Adipose tissue and skeletal muscle appear to be the key targets for TZDs.[119-122] TZD activation of PPAR-γ in skeletal muscle is associated with increased expression of lipoprotein lipase and GLUT4.[78,110] In this context, several studies have shown that treatment with TZDs increases adiponectin, rising the hypothesis that some of the anti-diabetic action of these drugs are due to the effects on adiponectin levels.

The influence of PPAR-γ polymorphisms on fat intake response has been investigated.[123-125] Cardona et al,[123] studied postprandial lipid metabolism in subjects classified according to their PPAR-γ and APOE genotypes, the corresponding genes being named *PPARG* and *APOE,* respectively. A variant of *PPARG, PPARG2,* contains an amino substitution of proline for alanine at codon 12 (Pro12Ala). This variant together with a non-E3/E3 *APOE* genotype was associated with a high risk for postprandial hypertriglyceridemia in patients with the MetS. It was observed that the Ala12 sequence variant is associated with a worse metabolic profile than Pro12 in relation to PPAR-γ expression and oxidative imbalance after the fat load.[124]

Recent genome-wide association studies have provided an important resource for furthering our understanding of T2DM disease mechanisms. Genes previously unsuspected of playing a role in diabetes are now implicated in the disease process. These include genes in cell cycling control (CDKN2A/2B, CDKAL1), transcription factors (TCF7L2, HHEX), and ion channels (SLC30A8). Two common variants alter diabetes risk through a primary effect on obesity. These variants are all associated with insulin-secretory defects in the general population and show little if any relationship to insulin resistance.[126]

PUFA and n-3 PUFA at high doses may increase oxidative stress, which may interact with diverse gene variants, as indicated above. Also n-3 PUFA are agonists of PPAR-γ, and this mechanism may explain some of the effects of n3 on insulin sensitivity. Therefore, research into the response of individuals presenting the Ala12 or Pro12 of the *PPARG2* to dietary n-3 LC-PUFA, or n-3 PUFA supplements is highly advisable. Similar nutrigenomic interaction should be explored as new polymorphisms are demonstrated to be implicated in the MetS.

Conclusions

The use of n-3 fatty acids should be considered within more global strategies, which include changes in lifestyle, such as adhering to a healthy Mediterranean type of diet and doing regular physical exercise. Reduction of dietary SFA and its partial replacement by MUFA and PUFA, including n-3 family, leads to an improvement in insulin sensitivity and associated metabolic abnormalities. There has been concern in the past about a possible deterioration in glucose homeostasis after intake of n-3 fatty acids in patients with T2DM, but these have been generally observed after using very high doses of n-3 PUFA supplements and the possible interacting effect of mercury ingestion has not been considered. Fish oil, a natural source of n-3 PUFA, has many physiological effects, indeed, it reduces insulin response to oral glucose without altering the glycaemic response in healthy humans; this is encouraging in the perspective of prevention of insulin resistance but further clinical and basic studies must be designed to confirm and complete our knowledge in this field. Finally, the nutrigenomic viewpoint should be included in all studies.

Acknowledgements

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