Endocrinol Nutr. 2015;62(7):338–349

ENDOCRINOLOGÍA Y NUTRICIÓN

www.elsevier.es/endo

REVIEW ARTICLE

The effect of polyunsaturated fatty acids on obesity through epigenetic modifications☆

Julián F. Hernando Boigues[a], Núria Mach[a,b,*]

[a] *Àrea de Ciències de la Salut, Institut Internacional de Postgrau, Universitat Oberta de Catalunya (UOC), Barcelona, Spain*
[b] *INRA, Animal Genetics and Integrative Biology Unit, Jouy-en-Josas, France*

Received 3 October 2014; accepted 17 March 2015
Available online 9 September 2015

KEYWORDS
PUFA;
microRNAs;
Epigenetics;
Obesity

Abstract
Background and purpose: In recent years it has been demonstrated that polyunsaturated fatty acids (PUFA) have anti-inflammatory and as regulators of lipid metabolism. However, the epigenomic mechanisms involved in these processes are not known in depth. The aim of this review was to describe the scientific evidence supports that regular consumption of PUFA may reduce obesity and overweight by altering epigenetic marks.
Material and methods: A search of recent publications was carried out in human clinical trials, as well as animal model and *in vitro* experiments.
Results: Exist a possible therapeutic effect of PUFAs on the prevention and development of obesity due to their ability to reversively modify the methylation of the promoters of genes associated with lipid metabolism and to modulate the activity of certain microRNAs.
Conclusions: A better knowledge and understanding of the PUFAs role in epigenetic regulation of obesity is possible with the current published results. The PUFAs may modulate the promotor epigenetic marks in several adipogenic genes and regulate the expression of several miRNAs.

PALABRAS CLAVE
Ácidos grasos poliinsaturados;
MicroARN;
Epigenética;
Obesidad

Efecto de los ácidos grasos poliinsaturados en la prevención de la obesidad a través de modificaciones epigenéticas

Resumen
Antecedentes y objetivo: En los últimos años se ha demostrado que los ácidos grasos poliinsaturados (AGPI) tienen efectos antiinflamatorios y como reguladores del metabolismo lipídico. No obstante, no se conocen en profundidad los mecanismos epigenómicos implicados en estos

☆ Please cite this article as: Hernando Boigues JF, Mach N. Efecto de los ácidos grasos poliinsaturados en la prevención de la obesidad a través de modificaciones epigenéticas. Endocrinol Nutr. 2015;62:338–349.

* Corresponding author.
E-mail address: nuria.mach@jouy.inra.fr (N. Mach).

2173-5093/

procesos. El objetivo de esta revisión fue describir las evidencias científicas que apoyan que el consumo regular de AGPI puede reducir la obesidad mediante modificaciones de las marcas epigenéticas.
Material y métodos: Se realizó una búsqueda de publicaciones recientes llevadas a cabo en ensayos clínicos en humanos, modelos animales o ensayos *in vitro*.
Resultados: Existe un posible efecto terapéutico de los AGPI sobre la prevención y desarrollo de la obesidad gracias a su capacidad de modificar reversiblemente la metilación de los promotores de genes asociados con el metabolismo lipídico y de modular la actividad de determinados microARN.
Conclusiones: Los resultados publicados hasta la fecha referentes al rol de los AGPI en la prevención de la obesidad contribuyen al mejor conocimiento y entendimiento de las modificaciones epigenéticas de la obesidad. Los AGPI han demostrado poder modificar epigenéticamente diferentes genes adipogénicos mediante la metilación de sus promotores o mediante la regulación de su interacción con diversos microARN.

Introduction

Overweight and obesity are defined as an abnormal or excess fat accumulation that may impair health.[1] This is a complex multifactorial disorder where both genetic and environmental factors interact. The body mass index (BMI) is the most commonly used tool for classifying overweight and obesity, and may be defined as the ratio between weight in kilograms and the square of height in meters (kg/m^2).[1] BMI values of 25 or higher represent overweight, while values of 30 or higher represent obesity.[1] This measure correlates well with body adiposity. Excess weight is associated with increased morbidity and mortality, including an increased risk of type 2 diabetes mellitus, atherosclerosis, high blood pressure, hyperlipidemia, osteoarthritis, sleep apnea syndrome, and some types of cancer.[2]

There is currently a pandemic of overweight and obesity which has been increasing for decades[2] and continues to increase.[3] A study of the worldwide population published in 2008 estimated that 23.2% of the adult population had overweight, and 9.8% obesity, which represents some 937 million and 396 million people with overweight and obesity respectively.[3] This study also predicted for 2030 an adult population of up to 2160 million people with overweight and 1120 million with obesity if the secular trends seen to date continue.[3] The situation is Spain is also worrying. A study published in 2011[4] reported a 34.2% prevalence of overweight in adults, with higher values in males (43.9%) as compared to females (25.7%). Obesity was reported in 13.6%, with no sex difference.[4] This growing prevalence of obesity is related to an increased prevalence of metabolic syndrome.[5] The definition of this syndrome, which is closely related to abdominal fat, usually refers to glucose intolerance, abdominal obesity, hypertension, and dyslipidemia that severely impair the health of those who suffer from it.[5,6] Obesity is therefore a significant public health problem, and involves high financial costs because of its associated comorbidities.[2] The worldwide financial burden of obesity has been estimated to range from 0.7% to 2.8% of all healthcare expenses, with a financial impact of 9.1% for overweight and obesity.[2] The most commonly accepted model for explaining human obesity is based on the interaction between genetic predisposition, metabolic abnormalities, and environmental factors such as sedentary lifestyles and unhealthy nutrition. Specifically, it has been estimated from twin, adoption, and familial studies that the genetic component causes approximately 40% of interindividual variability in obesity values.[7,8] More specifically, comparisons of twin studies with familial and adoption studies show that 60–90% of the BMI variance in the population may be explained by genetic effects.[9] Linkage and association studies have located multiple obesity *loci* along the genome.[10] The central role of lipid metabolism in obesity and overweight has led to extensive analysis of the genetic varieties of genes encoding for the proteins involved in the metabolic pathways of adipogenesis, energy intake, lipolysis, and energy expenditure. Thus, for example, polymorphisms in the apolipoprotein B[11] and A5,[11] CD36 (cluster of differentiation 36),[12] USF1 (upstream transcription factor 1),[13,14] FADS3 (fatty acid desaturase 3),[14] GCKR (glucokinase regulatory protein),[15] INSIG2 (insulin-induced gene 2),[16] NPP1 (ectonucleotide pyrophosphatase/phosphodiesterase 1),[17] FTO (fat mass and obesity-associated protein),[18] and CTNNBL1 (catenin beta like 1)[19] genes have been studied. More than 40 genetic variants associated with obesity and body fat distribution are currently known[20] (Table 1). However, these studies with genetic markers cannot fully account for the heritability of obesity. This may partly be due to the polygenetic nature of obesity, in which different variants of the DNA sequence have only a small effect. For this reason, a very large analysis population is required for detection.[10]

Another potential explanation is the existence of other forms of variation, such as epigenetic modifications and alterations.[20] Epigenetics may currently be defined as the heredity of DNA activity that does not depend on the sequence itself, but on chemical modifications in DNA and adjacent regulatory proteins.[21] The best known epigenetic marks include the addition of a methyl group to DNA in cytosine of the CpG dinucleotide.[21] These dinucleotides are abundant in the promoter regions of many genes. Hypermethylation is usually associated with decreased gene

Table 1 The 54 *loci* associated with phenotypes of anthropometric obesity.

Nearest gene(s)	Chromosome location	Phenotype	Associated SNP(d)	Function	Additional phenotypes
TBX15-WARS2	1p12	WHR	rs984222	Transcription factor involved in adipocytes and specific development of adipose depot	Involved in Cousin syndrome
PTBP2	1p21.3	BMI	rs1555543	–	
NEGR1	1p31	BMI	rs2815752, rs3101336	Neuron expansion	
TNNI3K	1p31.1	BMI	rs1514175	–	
DNM3-PIGC	1q24.3	WHR	rs1011731	Dominant negative mutations in DNM enzymes promote GLUT6 and GLUT8 transporters to the cell surface of adipocytes in rats	
SEC16B, RASAL2	1q25	BMI	rs10913469	–	
LYPLAL1; *ZC3H11B*	1q41	WHR	rs2605100	Encodes for protein believed to act as triglyceride lipase and increased in subcutaneous adipose tissue in obese patients	
SDCCAG8	1q43–q44	BMI	rs12145833	–	
FANCL	2p16.1	BMI	rs887912	–	
RBJ-ADCY3-POMC	2p23.3	BMI	rs713586	–	Rare POMC mutations cause obesity in humans
TMEM18	2p25	BMI	rs6548238, rs2867125, rs4854344, rs7561317, rs11127485	Neuron development	Associated with T2 diabetes
ZNRF3-KREMEN1	2q12.1	WHR	rs4823006	–	Protein Kremen1 forms a complex with LDL receptor-related protein 6
LRP1B	2q22.2	BMI	rs2890652	–	Deletions in LRP1B occur in several types of human cancer
GRB14	2q24.3	WHR	rs10195252	–	Associated with triglyceride and insulin levels. GRB14-deficient mice show increased weight
ADAMTS9	3p14.1	WHR	rs6795735	Important for spatial cell distribution in embryonic development	Associated with T2 diabetes

Table 1 (*Continued*)

Nearest gene(s)	Chromosome location	Phenotype	Associated SNP(d)	Function	Additional phenotypes
NISCH-STAB1	3p21.1	WHR	rs6784615	Interacts with insulin receptor substrate	
CADM2	3p21.1	BMI	rs13078807	–	
ETV5 (locus with 3 genes, stronger association in *ETV5*)	3q27	BMI	rs77647305	–	
Gene desert; *GNDA2* is one of the 3 close genes	4p13	BMI	rs10938397	–	Associated with T2 diabetes
SLC39A8	4q24	BMI	rs13107325	–	
FLJ35779	5q13.3	BMI	rs2112347	–	
ZNF608	5q23.2	BMI	rs4836133	–	
CPEB4	5q35.2	WHR	rs6861681	Regulates elongation of polyadenylation	
TFAP2B	6p12	WC, BMI	rs987237	–	
Locus containing *NCR3*, *AIF1* and *BAT2*	6p21	BMI	rs2844479, rs2260000, rs1077393	–	Associated with weight but not with BMI
VEGFA	6p21.1	WHR	rs6905288	Involved in vascular development. Key mediator in adipogenesis	VEGFA variants nominally associated with T2 diabetes
NUDT3-HMGA1	6p21.31	BMI	rs206936	–	
PRL	6p22.1–p21.3	BMI	rs4712652	–	
LY86	6p25.1	WHR	rs1294421	Plays a role in polysaccharide recognition	Associated with asthma
RSPOS	6q22.33	WHR	rs9491696	Promotes angiogenesis and vascular development	Oncogene in breast epithelial cells in mice
NFE2L3	7p15.2	WHR	rs1055144	–	
MSRA	8p23.1	WC, BMI	rs7826222, rs17150703	–	
LRRN6C	9p21.3	BMI	rs10968576	–	
PTER	10p12	BMI	rs10508503	–	
MTCH2 (locus with 14 genes)	11p11.2	BMI	rs10838738	Cell apoptosis	
BDNF (locus with 4 genes, stronger association near *BDNF*)	11p14	BMI	rs4074134, rs4923461, rs925946, rs10501087, rs6265	BDNF expression is regulated by nutritional status and MC4R signaling	Associated with T2DM Subjects with WAGR syndrome with BDNF deletion have BMI >95th percentile. BDNF knockdown in mouse hypothalamus causes hyperphagia and obesity

Table 1 (*Continued*)

Nearest gene(s)	Chromosome location	Phenotype	Associated SNP(d)	Function	Additional phenotypes
RPL27A	11p15.4	BMI	rs4929949	–	
ITPR2-SSPN	12p21.1	WHR	rs718314	–	Mice deficient in ITPR2 and ITPR3 exhibited hypoglycemia and thinness
HOXC13	12q13.13	WHR	rs1443512	Important transcription factor in spatial distribution and embryonic development	
FAIM2 (locus also contains BCDIN3D)	12q13	BMI	rs7138803	Apoptosis in adipocytes	
C12orf51	12q24	WHR	rs2074356	–	
MTIF3-GTF3A	13q12.2	BMI	rs4771122	–	
PRKD1	14q12	BMI	rs11847697	–	
NRXN3	14q31	WC, BMI	rs10146997	–	
MAP2K5	15q23	BMI	rs2241423	–	
SH2B1 (locus with 19–25 genes)	16p11.2	BMI	rs7498665, rs8049439, rs4788102, rs7498665	Neuron role in energy homeostasis	Sh2b1-null mice are obese and diabetic
GPRC5B	16p12.3	BMI	rs12444979	–	
MAF	16q22–q23	BMI	rs1424233	Transcription factor involved in adipogenesis and insulin-glucagon regulation	
FTO	16q22.2	BMI	rs9939609, rs6499640, rs8050136, rs3751812, rs7190492, rs8044769, rs1558902	Neuronal function associated with appetite control	Associated with T2 diabetes
NPC1	18q11.2	BMI	rs1805081	Intracellular lipid transport	NPC1-null mice show late onset weight loss and poor intake. NPC1 interferes with the signaling function of raft-associated insulin receptor
MC4R	18q22	BMI	rs17782313, rs12970134, rs17700144	Hypothalamic signaling	Haploinsufficiency in humans is associated with morbid obesity. MC4R-deficient mice show hyperphagia and obesity

Table 1 (*Continued*)

Nearest gene(s)	Chromosome location	Phenotype	Associated SNP(d)	Function	Additional phenotypes
KCTD15	19q13.11	BMI	rs11084753, rs29941	–	
QPTCL-GIPR	19q13.32	BMI	rs2287019	Encodes for incretin receptor	Associated with fasting and 2-h glucose
TMEM160	19q13.32	BMI	Rs3810291	–	
RPL27A	11p15.4	BMI	rs4929949	–	
ITPR2-SSPN	12p21.1	WHR	rs718314	–	Mice deficient in ITPR2 and ITPR3 exhibited hypoglycemia and thinness
HOXC13	12q13.13	WHR	rs1443512	Important transcription factor in spatial distribution and embryonic development	
FAIM2 (locus also contains BCDIN3D)	12q13	BMI	rs7138803	Apoptosis in adipocytes	
C12orf51	12q24	WHR	rs2074356	–	

WC: waist circumference; BMI: body mass index; POMC: WHR: waist/hip ratio.
Source: Data adapted from Herrera et al.[20]

expression (silencing); by contrast, hypomethylation is associated with increased expression.[22,23] The concept of genetic imprinting is related to the DNA methylation level. This concept describes the heredity of specific epigenetic information from one of the parents. Some genes acquire a maternal or paternal imprint during gametogenesis and, as a result, are widely expressed from a single allele during embryonal development and in adult tissues.[24] Defective genetic imprinting is associated with developmental disorders and clinical phenotypes, among which abnormal body weight is usually included.[24] A well known example is Prader–Willi syndrome, characterized by cognitive impairment and voracious and uncontrollable appetite, which is often associated with the development of severe obesity in the first six years of life.[24] An additional epigenetic mark studied is the modification of the proteins called histones. In addition to packaging DNA, histones play a very significant role in post-translational modifications of their amino acids (*e.g.* lysine acetylation, arginine methylation, serine phosphorylaton)[21] Other epigenetic marks under study are defined by the arrangement of high-order structures formed by DNA-histone complexes (the so-called nucleosomes) and the activity of non-coding RNAs such as microRNAs, interference RNAs, long-chain non-coding RNAs, and antisense RNAs, amongst others.[25,26] These non-coding RNAs regulate post-transcriptionally gene expression through their pairing to the 3′ untranslated region (3′ UTR) of messenger RNA.[27] For example, miR-33 and miR-122 control triglyceride metabolism and cholesterol biosynthesis in mouse liver, and suggest that their dysregulation is directly associated with the development of metabolic diseases such as obesity and metabolic syndrome.[28,29] The implication of long-chain non-coding RNAs (lncRNAs) in adipose tissue plasticity and the regulation of adipogenesis is also known.[30,31]

As previously reported, obesity is a multifactorial, polygenic disorder where genetic and epigenetic factors interact with environmental factors such as physical activity, alcohol, and smoking. However, nutrition is probably the most important factor.[32] In addition, epigenetic changes show a great plasticity and respond to environmental signals, including diet.[33] Because of the influence of maternal metabolism on embryo development during pregnancy, it has been suggested that the nutritional status of the mother during pregnancy may induce epigenetic dysfunctions in the newborn.[34–40] Although epigenoma involvement occurs at specific time periods, in the first stages of embryogenesis and infancy, intervention in adult age is also possible.[33] Exposure to diets rich or deficient in given nutrients for long time periods (years) has been seen to induce epigenetic changes with consequences for health and the risk of disease.[33] Thus, polyphenols exert their antilipidemic and antiatherogenic activity not only by regulating the expression of different genes associated with the immune system and energy metabolism, but also by inducing changes in the methylation pattern of CpG islands of DNA,[41,42] histone acetylation,[43] and the modulation of expression of some miRNAs[44] in adults. In this regard, Joven et al.[45] used hyperlipidemic mice with LDL receptor deficiency to assess the role of polyphenols in the prevention of metabolic disease through the regulation of expression of the hepatic microRNAs miR-103/107 and miR-122. In their results, they stressed that oral polyphenol administration reversed the changes

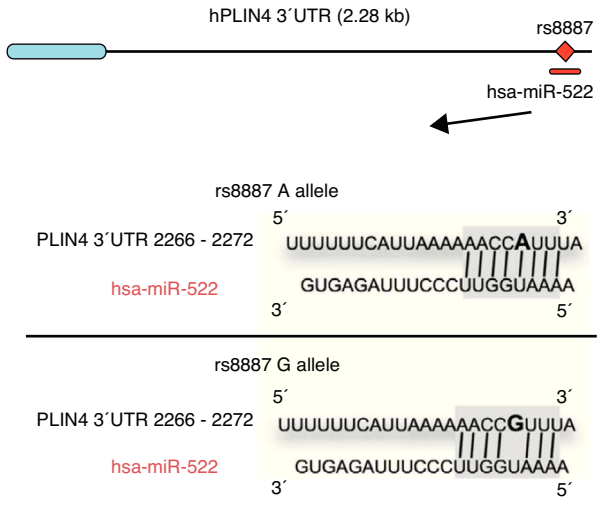

Figure 1 Minor A allele rs8887 creates a new miR-522 MRE in the PLIN4 3ÚTR gene. miR-522 diagram: PLIN4 3ÚTR sequences with the A or G allele. The miR-522 site is shaded gray, and variant rs8887 appears in bold.
Adapted from Richardson et al.[67]

caused in the non-specific microRNAs miR-103/107 after chronic polyphenol intake, along with the lack of response of the specific miRNA miR-122, and speculated about a potential implication of polyphenols in cell metabolism in the liver. They also postulated that the modulation of microRNA expression could be a significant additional mechanism of intervention in chronic diseases. Despite the foregoing, additional studies are required in humans to elucidate the epigenetic effects of polyphenols and other components such as long-chain PUFAs.

Omega-3 fatty acids (n-3) have been related to various properties and therapeutic uses in humans.[46–53] Thus, intake of the recommended amounts of n-3 compounds docosahexaenoic (DHA) and eicosapentaenoic acids decreases the risk of death and coronary diseases by preventing arrhythmia, the formation of prostaglandins and leukotriene precursors, the inhibition of inflammatory cytokines, the promotion of lipolysis, and fatty acid oxidation, as well as the inhibition of lipogenesis and a reduction in total triglycerides and very low density lipoproteins (VDVDLc). Diets with high n-3 contents have recently been seen to decrease the risk of the development of different types of cancer (*e.g.* colorectal and breast cancer) and their cell proliferation, among other properties.[54–58] The molecular processes associated with antilipidemic and antiatherogenic properties, as well as the anti-inflammatory and anti-cell development, of n-3 fatty acids result from their ability to regulate the expression of different genes associated with the immune system and energy metabolism,[59–62] or their epigenetic regulation capacity through the induction of changes in the methylation pattern of CpG islands of DNA,[63] and the modulation of expression of some miRNAs.[64–66] In this regard, for example, it has been reported that n-3 PUFAs modify the interaction between miR-522 and the 3′ UTR region of the perilipin 4 gene (PLIN4), resulting in a change in obesity-related phenotypes (Fig. 1).[67] However, few studies are available reporting the effect of the intake of different types of polyunsaturated fatty acids (PUFAs) on epigenetic modification and the resulting genetic expression. Consequently, there is a need to verify the public data and to illustrate the relationship between the intake of PUFAs, especially n-3, and epigenetic modifications. The purpose of this study was therefore to review the most recent studies on the effects of PUFA intake and the risk of obesity or overweight, in an attempt to elucidate the associated epigenetic mechanisms, especially DNA methylation and the role of non-coding RNAs.

Materials and methods

In this review, a search of recent publications was made in the following specialized electronic databases: NCBI, Elsevier Journal, Scielo, Science Direct, Springer Link. The results from studies conducted *in vitro*, in animal models, and in humans were collected. Reviews collecting and analyzing the effectiveness of PUFAs in certain treatments, such as antihypertensive and lipid lowering therapies, among others, were also included. Epigenetic concepts related to non-coding RNAs and chemical modifications in histones, obesity, high blood pressure, and atherosclerosis were also analyzed to describe in greater detail the potential epigenetic mechanisms of PUFAs. The following keywords were used: polyunsaturated fatty acids, histone acetylaton, DNA methylation, microRNA, epigenetics, obesity, overweight, and metabolic syndrome. A total of 84 articles were revised, including reviews. The articles selected were divided into the following categories: (1) generic articles on epigenetics, obesity, and PUFAs; (2) articles on the relationship between PUFA consumption and DNA methylation, histone acetylation, and non-coding RNA modulation.

Results and discussion

Few reports are available on the epigenetic effect of the intake of n-3 and n-6 PUFAs and their role in obesity control and prevention. Studies analyzing the effects of PUFAs on epigenetic modifications used in this review were grouped based on the type of epigenetic mark: (1) the addition of a methyl group to DNA at the cytosine in the CpG dinucleotide; (2) modification of the so-called histone proteins; (3) modification of non-coding RNA expression. The most relevant results and conclusions are specified for each of them.

As stated above, different demethylation waves occur during the first few days of embryo development, followed by increased *de novo* methylation in embryo and extraembryonic tissues such as the placenta.[68,69] Guo et al.[68] showed that the greatest demethylation wave is completed in the two-cell stage. Soon after this implantation, a remethylation wave occurs, and epigenetic patterns are established for the different cell types.[33,68] During pregnancy, there may be a first contact between the embryo and nutrients or secondary metabolites from the mother, influencing fetal epigenoma and increasing or decreasing the risk of developing some diseases. Kulkarni et al.[63] reported that supplementation with n-3 (45 g of fish oil and 25 g of soybean oil per kg of diet) to pregnant rats combined with excess folic and vitamin B_{12} deficiency increased DNA methylation in the placenta to control levels. Thus, decreased DNA methylation levels in rat placenta were reversed when the diet

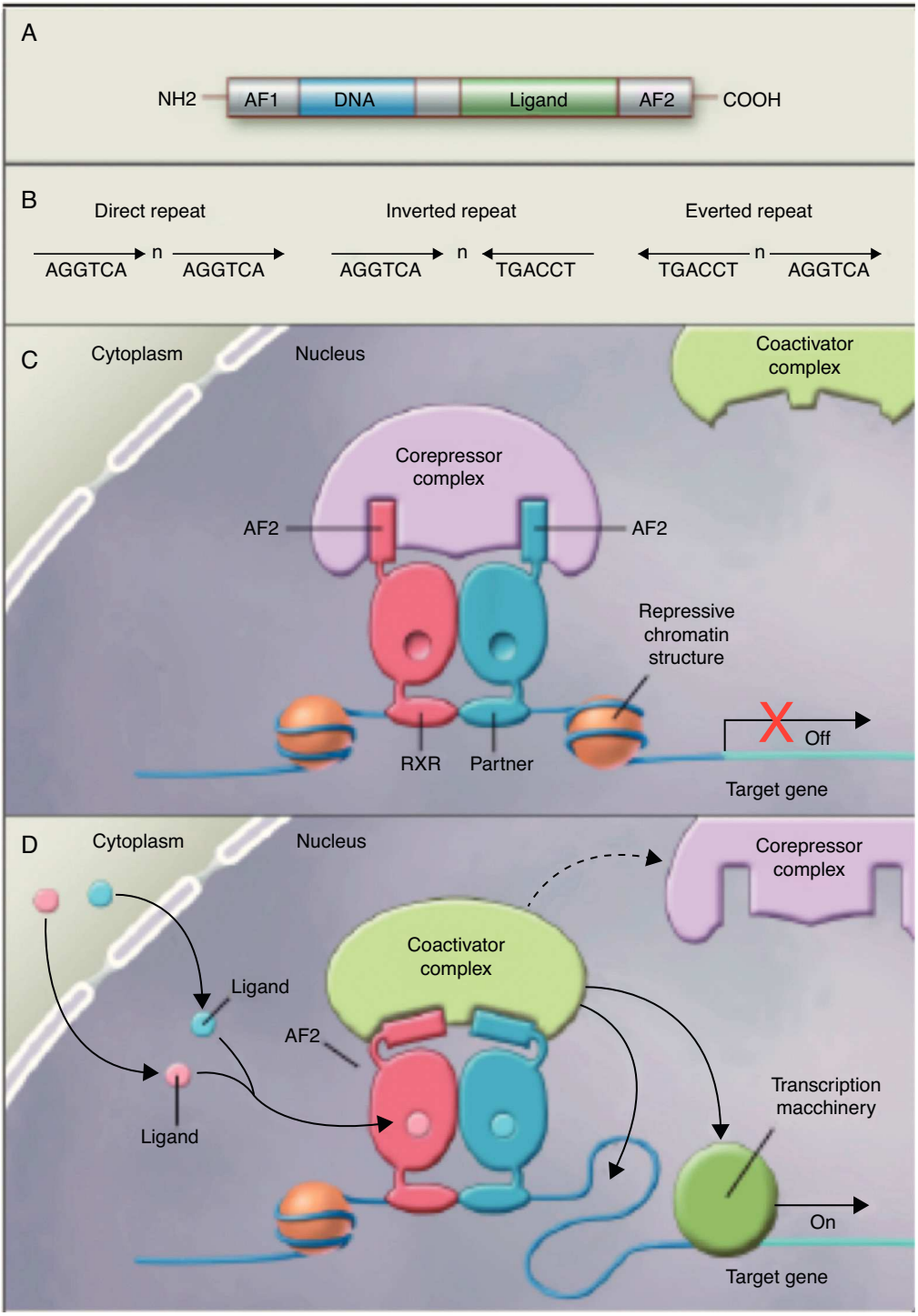

Figure 2 Nuclear receptors as ligand-dependent transcription factors. (A) Canonical structure of a nuclear response element (NRE) including the n N-terminal activation function (AF1), DNA binding, ligand binding, and C-terminal domains (AF2). (B) Number of nucleotides between the central elements (n) that confer additional specificity. (C, D) Heterodimer without and with ligand associated with the corepressor and coactivator complex.
Adapted from Shulman et al.[84]

was supplemented with DHA, showing that DHA levels play a very important role in determining placental methylation levels. These results are consistent with those obtained in studies in animal models where n-3 supplementation during pregnancy[70] or during the first days after birth[71] was able to prevent or reduce the adverse effects of fetal programming. In obese adult mice, Fan et al.[72] showed that the regulation of expression of leptin, leptin receptor, and the neuropeptide precursor proopiomelanocortin (POMC) genes was modified by diet supplementation with n-3

(35 g/kg of soybean oil; 17.5 g/kg of soybean oil and 17.5 g/kg of fish oil; 35 g/kg of fish oil for each of the three groups with no n-3 deficiency), but that the methylation of the promoters of those genes did not change.[72] Other studies conducted in animal models have shown that the effect of n-3 supplementation on DNA methylation depends on the gene and tissue studied, particularly during pregnancy and lactation.[63,73,74] Thus, Niculescu et al.[74] were able to show an association between the availability of α-linolenic acid (ALA; supplementation of 75,367 nmol/mg/day) during pregnancy and lactation in mice and changes in DNA methylation of the FADS2 gene (fatty acid desaturase 2) and intron number 1 in livers from dams and pups at the end of the lactation period. FADS2 is a desaturase enzyme that catalyzes the different steps in the biosynthetic pathway of long-chain PUFAs from linoleic acid (n-6) and ALA.[75] Moreover, this study suggested that maternal interaction with ALA during pregnancy and lactation could differentially alter n-3 and n-6 metabolism.[74] In humans, a recent study conducted in young women with overweight treated with a calorie restricted diet showed that supplementation with n-3 derived from fish oil (>1300 mg/day as 6 capsules daily) induced small epigenetic changes that decreased DNA methylation of the CD36 gene of mononuclear cells in blood after adjustment for the body weight of the women.[76]

No studies were found relating the effects of the intake of PUFAs and their metabolites to histone acetylation and the resultant chromatin remodeling, which is important for the expression of genes of the nuclear receptor superfamily associated with the control and development of obesity and lipid metabolism.[77] Although no studies are available on the subject, some authors[78] are coming round to the idea that long-chain PUFAs could make it possible to control the expression of PPAR (peroxisome proliferator-activated receptor gamma) and its target genes through sequential chromatin remodeling. In other words, PUFA intake could modify the multiprotein corepressor complex with histone deacetylase activity, modify chromatin remodeling, permitting transcription factor binding to its promoter, facilitating its transcription and the expression of all target genes, many of which are related to obesity (Fig. 2).

Finally, we report on studies showing the regulation of non-coding RNAs by PUFAs and its potential implications in obesity. The first example of a genetic variant that results in a binding site for a microRNA (miRNA) which influences the traits related to obesity through a gene–diet interaction modulated by n-3 PUFAs was recently shown.[67] miRNAs are small non-coding transcripts consisting of approximately 21–25 nucleotides. They play a determinant role in the regulation of genes associated with processes of cell differentiation and development, the proliferation and maintenance of homeostasis, amongst others. These miRNAs, associated with multienzyme complexes, are guided for the recognition of complementary sequences in the 3′ UTR or 5′ UTR region of mRNA.[79] Their interaction usually leads to mRNA degradation and translational repression, with a subsequent reduction in protein activity. Richardson et al.[67] investigated the relationship between 7 SNPs in the PLIN4 gene (rs8887, rs11673616, rs892158, rs7250947, rs8102428, rs1609717, rs884164) and obesity-related phenotypes from samples of subjects from two populations of European ancestry.[67] These authors conducted a meta-analysis which showed significant interactions between the rs8887 polymorphism for the minor A allele of the PLIN4 gene, the intake of n-3 PUFAs, and anthropometric measurements. PLIN4 is a protein of the PAT family with a great affinity for lipid storage[80] droplets, which have an influence on the risk of developing metabolic diseases.[81] The authors also reported that, at the structural level, the presence of the A allele in the 3′ untranslated region (3′ UTR) of the PLIN4 gene created a molecular recognition element (MRE) for miR-522, which did not occur in the case of the G allele (Fig. 1). Data provided by this study show that high n-3 intake may induce in allele A carriers decreased anthropometric values as compared to non-carriers, and specifically to homozygotes for the G allele, because there is no interaction in them between miRNA and the 3′ UTR region of the PLIN4 gene.[67] Decreased PLIN4 gene expression due to miR-522 may contribute to obesity-related phenotypes, but additional studies are required to confirm this, and to ascertain whether the proposed mechanism may be operative for other miRNAs. In another recent study, Baselga et al.[82] were able to counteract the dyslipidemic effect of two miRNAs by supplementing the diet of obese rats with proanthocyanidins and DHA. The miRNAs analyzed (miR-122 and miR-33a) are important regulators of lipid metabolism in the liver.[83] The study objective was to assess whether liver levels of miR-122 and miR-33a correlated with lipidemia induced by nutrition in different rat models. To do this, liver levels of both miRNAs were measured in dyslipidemic rats fed a cafeteria diet (CD) with no supplementation and rats fed a CD supplemented with proanthocyanidins or DHA. The CD was shown to increase miR-122 and miR-33a levels in the liver. By contrast, levels of both miRNAs were reversed in rats with DHA supplementation, with an even greater reduction in rats supplemented with both compounds (proanthocyanidins and DHA). With regard to the lipid profile, long-term treatment with proanthocyanidins improved the atherogenic index altered by CD, normalizing plasma triglyceride (TG) and LDL levels, and also decreased total lipid and TG levels in the liver. By contrast, rats fed CD supplemented with DHA showed a normalization of plasma total cholesterol and LDL levels, but the lipid content in the liver was not affected. The concomitant administration of both treatments (polyphenols and DHA) had a lipid-lowering effect, with decreases in liver and plasma levels similar to those achieved by individual treatments alone. The authors concluded that their effect was complementary, rather than synergistic or additive, but further studies are needed to elucidate the mechanism by which proanthocyanidins and DHA repress miR-122 and miR-33a.[82]

Conclusions

PUFA intake has been associated with different therapeutic properties. Specifically, based on the results of this review, we conclude that PUFA intake may control the parameters related to obesity through different epigenetic mechanisms. The early results suggest that PUFAs are able to reversibly modify the methylation of adipogenic gene promoters and, thus, their expression. This is a remarkable result, because epigenetic changes may be one of the weak points in the

development of obesity, as we can inactivate or activate epigenetically inactivated genes using adequate nutrients. There is currently no information on genetic modulation through alternative epigenetic mechanisms, such as histone modifications. However, according to the early results, the potential association between PUFAs and the repression of the expression of genes associated with lipid metabolism by miRNAs is starting to become evident in animal models.

The results published to date do not allow us to determine a dose of PUFAs in terms of its therapeutic properties. However, the results do represent interesting findings which should be thoroughly analyzed, because understanding of the distribution and function of PUFAs in obese patients may be helpful in achieving effective treatment. Continued research in the field of alternative non-drug treatments, such as functional foods, is also required. Only a limited number of PUFAs have been tested to date, and since the effects of the different components are not equivalent, the results cannot be generalized. Future large scale studies with control of doses, active components, bioavailability, and other critical variables, such as genetic background, will therefore be crucial for providing the scientific evidence required to ascertain the epigenetic modifications induced by PUFAs and their contribution to obesity development and prevention.

Conflicts of interest

The authors state that they have no conflicts of interest.

References

1. Morgen CS, Sorensen TI. Obesity, global trends in the prevalence of overweight and obesity. Nat Rev Endocrinol. 2014;10:513–4.
2. Shamseddeen H, Getty JZ, Hamdallah IN, Ali MR. Epidemiology and economic impact of obesity and type 2 diabetes. Surg Clin N Am. 2011;91:1163–72, vii.
3. Kelly T, Yang W, Chen CS, Reynolds K, He J. Global burden of obesity in 2005 and projections to 2030. Int J Obes (Lond). 2008;32:1431–7.
4. Rodriguez-Rodriguez E, Lopez-Plaza B, Lopez-Sobaler AM, Ortega RM. Overweight and obesity among Spanish adults. Nutr Hosp. 2011:355–63 [in Spanish].
5. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet. 2005;365:1415–28.
6. Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation. 2004;109:433–8.
7. Williams SM. Endophenotypes, heritability, and underlying complexity in hypertension. Am J Hypertens. 2010;23:819.
8. Wardle J, Carnell S, Haworth CM, Plomin R. Evidence for a strong genetic influence on childhood adiposity despite the force of the obesogenic environment. Am J Clin Nutr. 2008;87:398–404.
9. Hinney A, Vogel CI, Hebebrand J. From monogenic to polygenic obesity: recent advances. Eur Child Adolesc Psychiatry. 2010;19:297–310.
10. Thorleifsson G, Walters GB, Gudbjartsson DF, Steinthorsdottir V, Sulem P, Helgadottir A, et al. Genome-wide association yields new sequence variants at 7 loci that associate with measures of obesity. Nat Genet. 2009;41:18–24.
11. Johansen CT, Wang J, Lanktree MB, Cao H, McIntyre AD, Ban MR, et al. Excess of rare variants in genes identified by genome-wide association study of hypertriglyceridemia. Nat Genet. 2010:684–7.
12. Keller KL, Liang LC, Sakimura J, May D, van Belle C, Breen C, et al. Common variants in the CD36 gene are associated with oral fat perception, fat preferences, and obesity in African Americans. Obesity (Silver Spring). 2012;20:1066–73.
13. Pajukanta P, Lilja HE, Sinsheimer JS, Cantor RM, Lusis AJ, Gentile M, et al. Familial combined hyperlipidemia is associated with upstream transcription factor 1 (USF1). Nat Genet. 2004;36:371–6.
14. Plaisier CL, Horvath S, Huertas-Vazquez A, Cruz-Bautista I, Herrera MF, Tusie-Luna T, et al. A systems genetics approach implicates USF1, FADS3, and other causal candidate genes for familial combined hyperlipidemia. PLoS Genet. 2009;5:e1000642.
15. Santoro N, Zhang CK, Zhao H, Pakstis AJ, Kim G, Kursawe R, et al. Variant in the glucokinase regulatory protein (GCKR) gene is associated with fatty liver in obese children and adolescents. Hepatology. 2012;55:781–9.
16. Hotta K, Nakamura M, Nakata Y, Matsuo T, Kamohara S, Kotani K, et al. INSIG2 gene rs7566605 polymorphism is associated with severe obesity in Japanese. J Hum Genet. 2008;53:857–62.
17. Meyre D, Bouatia-Naji N, Tounian A, Samson C, Lecoeur C, Vatin V, et al. Variants of ENPP1 are associated with childhood and adult obesity and increase the risk of glucose intolerance and type 2 diabetes. Nat Genet. 2005;53:863–7.
18. Moleres A, Ochoa MC, Rendo-Urteaga T, Martinez-Gonzalez MA, Azcona San Julian MC, Martinez JA, et al. Dietary fatty acid distribution modifies obesity risk linked to the rs9939609 polymorphism of the fat mass and obesity-associated gene in a Spanish case–control study of children. Br J Nutr. 2012;107:533–8.
19. Liu YJ, Liu XG, Wang L, Dina C, Yan H, Liu JF, et al. Genome-wide association scans identified CTNNBL1 as a novel gene for obesity. Hum Mol Genet. 2008;107:1803–13.
20. Herrera BM, Keildson S, Lindgren CM. Genetics and epigenetics of obesity. Maturitas. 2011;69:41–9.
21. Esteller M. Epigenetics in cancer. N Engl J Med. 2008;358:1148–59.
22. Marti A, Ordovas J. Epigenetics lights up the obesity field. Obesity Facts. 2011;4:187–90.
23. Klose RJ, Bird AP. Genomic DNA methylation: the mark and its mediators. Trends Biochem Sci. 2006;31:89–97.
24. Stoger R. Epigenetics and obesity. Pharmacogenomics. 2008;9:1851–60.
25. Castel SE, Martienssen RA. RNA interference in the nucleus: roles for small RNAs in transcription, epigenetics and beyond. Nat Rev Genet. 2013;14:100–12.
26. Liu N, Pan T. RNA epigenetics. Transl Res. 2014.
27. Mattick JS, Makunin IV. Non-coding RNA. Hum Mol Genet. 2006, 15 Spec No 1:R17-R29.
28. Heneghan HM, Miller N, Kerin MJ. Role of microRNAs in obesity and the metabolic syndrome. Obes Res. 2010;11:354–61.
29. Rottiers V, Naar AM. MicroRNAs in metabolism and metabolic disorders. Nat Rev Mol Cell Biol. 2012;13:239–50.
30. Xu B, Gerin I, Miao H, Vu-Phan D, Johnson CN, Xu R, et al. Multiple roles for the non-coding RNA SRA in regulation of adipogenesis and insulin sensitivity. PLoS ONE. 2010;5:e14199.
31. Sun L, Goff LA, Trapnell C, Alexander R, Lo KA, Hacisuleyman E, et al. Long noncoding RNAs regulate adipogenesis. Proc Natl Acad Sci U S A. 2013;110:3387–92.
32. Ordovas JM. Genotype–phenotype associations: modulation by diet and obesity. Obesity (Silver Spring). 2008;16 Suppl 3:S40–6.
33. Jimenez-Chillaron JC, Diaz R, Martinez D, Pentinat T, Ramon-Krauel M, Ribo S, et al. The role of nutrition on epigenetic modifications and their implications on health. Biochimie. 2012;94:2242–63.

34. Lillycrop KA, Burdge GC. Epigenetic changes in early life and future risk of obesity. Int J Obes (Lond). 2011;35:72–83.
35. Rhee KE, Phelan S, McCaffery J. Early determinants of obesity: genetic, epigenetic, and in utero influences. Int J Pediatr. 2012;2012:463850.
36. Martinez JA, Cordero P, Campion J, Milagro FI. Interplay of early-life nutritional programming on obesity, inflammation and epigenetic outcomes. Proc Nutr Soc. 2012;71:276–83.
37. Seki Y, Williams L, Vuguin PM, Charron MJ. Minireview epigenetic programming of diabetes and obesity: animal models. Endocrinology. 2012;153:1031–8.
38. Vucetic Z, Carlin JL, Totoki K, Reyes TM. Epigenetic dysregulation of the dopamine system in diet-induced obesity. J Neurochem. 2012;120:891–8.
39. Lavebratt C, Almgren M, Ekstrom TJ. Epigenetic regulation in obesity. Int J Obes (Lond). 2012;36:757–65.
40. Milagro FI, Mansego ML, de Miguel C, Martinez JA. Dietary factors, epigenetic modifications and obesity outcomes: progresses and perspectives. Mol Aspects Med. 2013;34:782–812.
41. Kato K, Long NK, Makita H, Toida M, Yamashita T, Hatakeyama D, et al. Effects of green tea polyphenol on methylation status of RECK gene and cancer cell invasion in oral squamous cell carcinoma cells. Br J Cancer. 2008;99:647–54.
42. Fang MZ, Wang Y, Ai N, Hou Z, Sun Y, Lu H, et al. Tea polyphenol (-)-epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines. Cancer Res. 2003;63:7563–70.
43. Ruiz PA, Braune A, Holzlwimmer G, Quintanilla-Fend L, Haller D. Quercetin inhibits TNF-induced NF-kappaB transcription factor recruitment to proinflammatory gene promoters in murine intestinal epithelial cells. J Nutr. 2007;137:1208–15.
44. Blade C, Baselga-Escudero L, Salvado MJ, Arola-Arnal A. miRNAs, polyphenols, and chronic disease. Mol Nutr Food Res. 2013;57:58–70.
45. Joven J, Espinel E, Rull A, Aragones G, Rodriguez-Gallego E, Camps J, et al. Plant-derived polyphenols regulate expression of miRNA paralogs miR-103/107 and miR-122 and prevent diet-induced fatty liver disease in hyperlipidemic mice. Biochim Biophys Acta. 2012;1820:894–9.
46. Guermouche B, Soulimane-Mokhtari NA, Bouanane S, Merzouk H, Merzouk S, Narce M. Effect of dietary n-3 polyunsaturated fatty acids on oxidant/antioxidant status in macrosomic offspring of diabetic rats. Biomed Res Int. 2014;2014:368107.
47. Li K, Huang T, Zheng J, Wu K, Li D. Effect of marine-derived n-3 polyunsaturated fatty acids on C-reactive protein interleukin 6 and tumor necrosis factor alpha: a meta-analysis. PLOS ONE. 2014;9:e88103.
48. Roca-Rodriguez MM, Garcia-Almeida JM, Lupianez-Perez Y, Rico JM, Toledo M, Alcaide-Torres J, et al. Effect of a specific supplement enriched with n-3 polyunsaturated fatty acids on markers of inflammation, oxidative stress and metabolic status of ear, nose and throat cancer patients. Oncol Rep. 2014;31:405–14.
49. Andersen AD, Ludvig SE, Damsgaard CT, Pulkkinen P, Finnila M, Mu H, et al. The effect of fatty acid positioning in dietary triacylglycerols and intake of long-chain n-3 polyunsaturated fatty acids on bone mineral accretion in growing piglets. Prostaglandins Leukot Essent Fatty Acids. 2013;89:235–40.
50. Rodriguez G, Iglesia I, Bel-Serrat S, Moreno LA. Effect of n-3 long chain polyunsaturated fatty acids during the perinatal period on later body composition. Br J Nutr. 2012;107 Suppl 2:S117–28.
51. Ibrahim A, Mbodji K, Hassan A, Aziz M, Boukhettala N, Coeffier M, et al. Anti-inflammatory and anti-angiogenic effect of long chain n-3 polyunsaturated fatty acids in intestinal microvascular endothelium. Clin Nutr. 2011;30:678–87.
52. Maaloe T, Schmidt EB, Svensson M, Aardestrup IV, Christensen JH. The effect of n-3 polyunsaturated fatty acids on leukotriene B(4) and leukotriene B(5) production from stimulated neutrophil granulocytes in patients with chronic kidney disease. Prostaglandins Leukot Essent Fatty Acids. 2011;85:37–41.
53. Zeghichi-Hamri S, de Lorgeril M, Salen P, Chibane M, de Leiris J, Boucher F, et al. Protective effect of dietary n-3 polyunsaturated fatty acids on myocardial resistance to ischemia-reperfusion injury in rats. Nutr Res. 2010;30:849–57.
54. Narayanan BA, Narayanan NK, Simi B, Reddy BS. Modulation of inducible nitric oxide synthase and related proinflammatory genes by the omega-3 fatty acid docosahexaenoic acid in human colon cancer cells. Cancer Res. 2003;63:972–9.
55. Dyari HR, Rawling T, Bourget K, Murray M. Synthetic omega-3 epoxyfatty acids as antiproliferative and pro-apoptotic agents in human breast cancer cells. J Med Chem. 2014;57:7459–64.
56. Fukui M, Kang KS, Okada K, Zhu BT. EPA omega-3 fatty acid, induces apoptosis in human pancreatic cancer cells: Role of ROS accumulation, caspase-8 activation, and autophagy induction. J Cell Biochem. 2013;114:192–203.
57. Brown I, Wahle KW, Cascio MG, Smoum-Jaouni R, Mechoulam R, Pertwee RG, et al. Omega-3 N-acylethanolamines are endogenously synthesised from omega-3 fatty acids in different human prostate and breast cancer cell lines. Prostaglandins Leukot Essent Fatty Acids. 2011;85:305–10.
58. Sun H, Hu Y, Gu Z, Owens RT, Chen YQ, Edwards IJ. Omega-3 fatty acids induce apoptosis in human breast cancer cells and mouse mammary tissue through syndecan-1 inhibition of the MEK-Erk pathway. Carcinogenesis. 2011;32:1518–24.
59. Farahbakhsh-Farsi P, Djalali M, Koohdani F, Saboor-Yaraghi AA, Eshraghian MR, Javanbakht MH, et al. Effect of omega-3 supplementation versus placebo on acylation stimulating protein receptor gene expression in type 2 diabetics. J Diabetes Metab Disord. 2014;13:1.
60. Aktas H, Halperin JA. Translational regulation of gene expression by omega-3 fatty acids. J Nutr. 2004;134:2487–91.
61. Price PT, Nelson CM, Clarke SD. Omega-3 polyunsaturated fatty acid regulation of gene expression. Curr Opin Lipidol. 2000;11:3–7.
62. Oh DY, Talukdar S, Bae EJ, Imamura T, Morinaga H, Fan W, et al. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. Cell. 2010;142:687–98.
63. Kulkarni A, Dangat K, Kale A, Sable P, Chavan-Gautam P, Joshi S. Effects of altered maternal folic acid vitamin B_{12} and docosahexaenoic acid on placental global DNA methylation patterns in Wistar rats. PLoS ONE. 2011;6:e17706.
64. Recchiuti A, Krishnamoorthy S, Fredman G, Chiang N, Serhan CN. MicroRNAs in resolution of acute inflammation: identification of novel resolvin D1-miRNA circuits. FASEB J. 2011;25:544–60.
65. Baselga-Escudero L, Arola-Arnal A, Pascual-Serrano A, Ribas-Latre A, Casanova E, Salvado MJ, et al. Chronic administration of proanthocyanidins or docosahexaenoic acid reverses the increase of miR-33 a and miR-122 in dyslipidemic obese rats. PLOS ONE. 2013;8:e69817.
66. Cirera S, Birck M, Busk PK, Fredholm M. Expression profiles of miRNA-122 and its target CAT1 in minipigs (Sus scrofa) fed a high-cholesterol diet. Comp Med. 2010;60:136–41.
67. Richardson K, Louie-Gao Q, Arnett DK, Parnell LD, Lai CQ, Davalos A, et al. The PLIN4 variant rs8887 modulates obesity related phenotypes in humans through creation of a novel miR-522 seed site. PLoS ONE. 2011;6:e17944.
68. Guo H, Zhu P, Yan L, Li R, Hu B, Lian Y, et al. The DNA methylation landscape of human early embryos. Nature. 2014;511:606–10.
69. Geiman TM, Muegge K. DNA methylation in early development. Mol Reprod Dev. 2010;77:105–13.
70. Grenier E, Ziv E, Delvin E, Leduc L, Spahis S, Lafond J, et al. n-3 fatty acids on utero programming of insulin resistance NASH and hyperlipidemia in *Psammomys obesus*. FASEB J. 2008:22.

71. Wyrwoll CS, Mark PJ, Mori TA, Puddey IB, Waddell BJ. Prevention of programmed hyperleptinemia and hypertension by posnatal dietary omega-3 fatty acids. Endocrinology. 2006;147:599–606.
72. Fan C, Liu X, Shen W, Deckelbaum RJ, Qi K. The regulation of leptin receptor and pro-opiomelanocortin expression by n-3 PUFAs in diet-induced obese mice is not related to the methylation of their promoters. Nutr Metab (Lond). 2011;8:31.
73. Hoile SP, Irvine NA, Kelsall CJ, Sibbons C, Feunteun A, Collister A, et al. Maternal fat intake in rats alters 20:4 n-6 and 22:6 n-3 status and the epigenetic regulation of Fads2 in offspring liver. J Nutr Biochem. 2013;24:1213–20.
74. Niculescu MD, Lupu DS, Craciunescu CN. Perinatal manipulation of alpha-linolenic acid intake induces epigenetic changes in maternal and offspring livers. FASEB J. 2013;27:350–8.
75. Chilton FH, Murphy RC, Wilson BA, Sergeant S, Ainsworth H, Seeds MC, et al. Diet-gene interactions and PUFA metabolism: a potential contributor to health disparities and human diseases. Nutrients. 2014;6:1993–2022.
76. Do Amaral CL, Milagro FI, Curi R, Martinez JA. DNA methylation pattern in overweight women under an energy-restricted diet supplemented with fish oil. Biomed Res Int. 2014;2014:675021.
77. Dilworth FJ, Chambon P. Nuclear receptors coordinate the activities of chromatin remodeling complexes and coactivators to facilitate initiation of transcription. Oncogene. 2001;20:3047–54.
78. Eeckhoute J, Oger F, Staels B, Lefebvre P. Coordinated regulation of PPAR gamma expression and activity through control of chromatin structure in adipogenesis and obesity. PPAR Res. 2012;2012, 164140, Epub 2012 Sep 6.
79. Filipowicz W, Jaskiewicz L, Kolb FA, Pillai RS. Post-transcriptional gene silencing by siRNAs and miRNAs. Curr Opin Struct Biol. 2005;15:331–41.
80. Kimmel AR, Brasaemle DL, McAndrews-Hill M, Sztalryd C, Londos C. Adoption of PERILIPIN as a unifying nomenclature for the mammalian PAT-family of intracellular lipid storage droplet proteins. J Lipid Res. 2010;51:468–71.
81. Greenberg AS, Coleman RA, Kraemer FB, McManaman JL, Obin MS, Puri V, et al. The role of lipid droplets in metabolic disease in rodents and humans. J Clin Invest. 2011;121:2102–10.
82. Baselga-Escudero L, Arola-Arnal A, Pascual-Serrano A, Ribas-Latre A, Casanova E, Salvado MJ, et al. Chronic administration of proanthocyanidins or docosahexaenoic acid reverses the increase of miR-33a and miR-122 in dyslipidemic obese rats. PLOS ONE. 2013:8.
83. Bommer GT, MacDougald OA. Regulation of lipid homeostasis by the bifunctional SREBF2-miR33a locus. Cell Metab. 2011;13:241–7.
84. Shulman AI, Mangelsdorf DJ. Retinoid x receptor heterodimers in the metabolic syndrome. N Engl J Med. 2005;353:604–15.