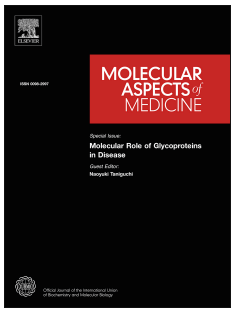
Accepted Manuscript



Of mice and humans *through the looking glass*: "Reflections" on epigenetics of lipid metabolism

Raffaella Longo, Alessandra Ferrari, Monica Zocchi, Maurizio Crestani

PII: S0098-2997(16)30114-5

DOI: 10.1016/j.mam.2017.01.005

Reference: JMAM 684

To appear in: *Molecular Aspects of Medicine*

Received Date: 13 December 2016

Revised Date: 18 January 2017

Accepted Date: 21 January 2017

Please cite this article as: Longo, R., Ferrari, A., Zocchi, M., Crestani, M., Of mice and humans *through the looking glass*: "Reflections" on epigenetics of lipid metabolism, *Molecular Aspects of Medicine* (2017), doi: 10.1016/j.mam.2017.01.005.

Of mice and humans *through the looking glass*: "reflections" on epigenetics of lipid metabolism

Raffaella Longo, Alessandra Ferrari[1], Monica Zocchi and Maurizio Crestani

Dipartimento di lScienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, via Balzaretti, 9 - 20133 Milano, Italia

Authors' e-mail: raffaella.longo@unimi.it, alessandra.ferrari1@unimi.it, monica.zocchi@unimi.it, maurizio.crestani@unimi.it

[1]Present address:

University of California, Irvine
Department of Biological Chemistry
324 Sprague Hall
Mail Code: 1700
Irvine, CA 92697, USA
e-mail: a.ferrari@uci.edu

Corresponding author

Maurizio Crestani, PhD
Professor, Biochemistry
Dipartimento di Scienze Farmacologiche e Biomolecolari - DiSFeB
Università degli Studi di Milano
via Balzaretti, 9
20133 Milano
Italia
ph: +39 02 50318393/1
e-mail: Maurizio.Crestani@unimi.it

Abstract

Over the past decade, epigenetics has emerged as a new layer of regulation of gene expression. Several investigations demonstrated that nutrition and lifestyle regulate lipid metabolism by influencing epigenomic remodeling. Studies on animal models highlighted the role of epigenome modifiers in specific metabolic contexts and established clear links between dysregulation of epigenetic mechanisms and metabolic dysfunction. The relevance of findings in animal models has been translated to humans, as epigenome-wide association studies (EWAS) deeply investigated the relationship between lifestyle and epigenetics in human populations. In this review, we will provide an outlook of recent studies addressing the link between epigenetics and lipid metabolism, by comparing results obtained in animal models and in human subjects.

Keywords: Epigenetics, chromatin, non-coding RNA, histones, lipid metabolism, nutrition

1. Introduction

All the living organisms constantly communicate with the environment. Both unicellular organisms and cells within a multicellular organism must be ready to adapt to several perturbations occurring in the external world (Turner 2009). The capacity to rapidly sense, integrate signals and elaborate a response is a signature of the plasticity and the robustness of a biological system (Kitano 2007). All the external stimuli and the environmental changes influence the cell behavior through the activation of specific cellular programs, resulting in rapid and stable alterations in gene expression. The epigenetic machinery plays an important role in the establishment of peculiar patterns of gene expression and hence in the regulation of physiological responses. Epigenetics include all the mechanisms that heritably change the transcriptome by modifying chromatin structure without variations in the DNA sequence (figure 1A). These mechanisms make DNA locally more packaged or accessible to transcriptional factors and RNA polymerases (i.e. eu-/heterochromatin

respectively). For this reason, chromatin is much more than a neutral system for packaging and condensing genomic DNA, but it is a highly dynamic platform that can be modified at different levels. The combination of different chromatin modifications constitutes the epigenetic code.

1.1 DNA methylation

The first layer of the epigenetic landscape architecture is DNA methylation: this covalent modification occurs at cytosine residues located at 5' of guanine residues ("CpG islands") and is mediated by DNA methyltransferase enzymes (Dnmt). In general, an exclusivity between active and "open" chromatin structures and DNA methylation exists (Weber et al. 2007). In fact, in mammalian genomes, the CG rich regions are prevalently unmethylated, for example high CpG-content promoters (HCPs) activate the expression of genes essential for cellular functions and are active by default (i.e. housekeeping genes promoters). On the contrary, the CG poor chromatin, such as low CpG-content promoters (LCPs), is methylated and seems to be selectively activated, for example by specific transcription factors.

1.2 Histone modifications

The second layer of the epigenome architecture is the "histone code", a combination of a variety of post-translational modifications of histones. In contrast to DNA that undergoes only methylation, histone tails can be modified by methylation, acetylation, phosphorylation, biotinylation, ubiquitination, sumoylation and ADP-ribosylation. Lysine residues in the histone tails can be either mono-, di- and tri- methylated or acetylated, meanwhile arginine residues can be only mono- or di-methylated. The level of histone acetylation is regulated by histone acetyltransferases (HAT) and histone deacetylases (HDAC). In contrast, regulation of histone methylation relies on histone methyltransferases (HMT) and demethylases (HDM) (for a complete review refer to (Barth and Imhof 2010; V. W. Zhou, Goren, and Bernstein 2011; J. Zhou and Troyanskaya 2016).

The diverse combination of different histone modifications in the same locus can provide a particular output in term of modulation of gene expression. Among the most important histone marks associated with an active transcription there are histone 3 lysine 4 tri-methylation (H3K4me3) at promoters, histone 3 lysine 4 mono and di-methylation (H3K4me1/me2) and histone 3 lysine 27 acetylation (H3K27ac) at enhancers, histone 3 lysine 36 tri-methylation (H3K36me3) and histone 3 lysine 79 tri-methylation (H3K79me3) along the gene bodies. On the contrary, among the most significant silenced chromatin marks there are histone 3 lysine 9 tri-methylation (H3K9me3), histone 3 lysine 27 di- and tri-methylation (H3K27me2/me3), histone 4 lysine 27 di- and tri-methylation (H4K27me2/me3) and histone 4 lysine 20 tri-methylation (H4K20me3).

1.3 Non Coding RNAs (ncRNAs)

Other important epigenetic regulators are non-coding RNAs (ncRNAs) (figure 1B) (for detailed review see Sadakierska-chudy & Małgorzata 2015). Among all, the class of long non-coding RNAs (lncRNAs > 200 nt in lenght) is the least characterized and their physiological role is still largely unknown. Recent investigations indicate that lncRNAs could play critical roles in essential physiological processes, such as nutrient sensing and maintenance of metabolic homeostasis. Most lncRNAs are localized in the nucleus and seem to be involved in the regulation of chromatin state. In fact, they can guide chromatin-modifying complexes to specific genomic loci and it has been demonstrated that lncRNAs recruit chromatin-remodeling complexes to specific chromatin loci (Moran, Perera, and Khalil 2012).

Another class of ncRNAs is the one of microRNAs (miRNAs). They are small single stranded RNA with 18-25 nucleotides in length that regulate mRNA degradation or protein translation. miRNAs can positively or negatively regulate expression of target genes (Ambros, 2001, Lewis et al., 2003), by inhibiting translation or by increasing degradation of target mRNA.

Epigenetic regulation mediated by miRNAs required the RNA-induced silencing complex (RISC), which is driven by miRNA on the target sequence via complementary base pairing. Interestingly it

has been demonstrated that aberrant expression of miRs correlates with the onset of several metabolic diseases, including obesity, insulin resistance, type 2 diabetes mellitus, and fatty liver disease (Lynn 2009; Hulsmans, Keyzer, and Holvoet 2016). Recently, many studies are combining these research areas to contribute to the advance of knowledge and, possibly, to identify new strategies of therapeutic intervention.

1.4 Nutrients influence epigenetic control of gene expression

It is now well known that epigenetic mechanisms not only regulate several important physiological processes such as embryonic development and aging, but they also play a central role in pathological processes such as cancer, inflammation, obesity, insulin resistance, type 2 diabetes mellitus, cardiovascular, neurodegenerative and immune diseases. It is likewise widely known that nutrients can modify pathophysiological processes by changing gene expression patterns through modifications of the epigenetic landscape resulting from alteration of metabolite levels (rev. in Lu & Thompson 2012; Katada et al. 2012; Keating & El-Osta 2015). At this regard, nutrients can influence epigenome either by directly regulating enzymes that catalyze DNA methylation or histone modifications or by altering the levels of metabolic intermediates necessary for enzyme reactions (Choi and Friso 2010; Jang and Serra 2014). In fact, all chromatin-modifying enzymes need coenzymes, such as ATP, Acetyl-CoA and S-adenosylmethionine (SAM) that function as phosphate, acetyl and methyl group donors. The level of these metabolites changes with the metabolism of energy-rich substrates such as carbohydrates and fatty acids present in diet (Martinez-Pastor, Cosentino, and Mostoslavsky 2013).

An important class of enzymes whose activity is directly dependent from the energetic status of the cell is the class III HDACs, also known as sirtuins (SIRT) (for details see review articles Ferrari et al. 2012; Ye et al. 2016). Sirtuins are NAD^+-dependent deacetylases, which cleave NAD^+ to create an ADP-ribose acceptor for the acetyl group, and hence their activity is sensitive to changes in the intracellular NAD^+/NADH ratio. Mammalian sirtuins have been connected to a wide range of

processes that encompass cellular stress resistance, genomic stability, tumorigenesis and they also play a central role in glucose and lipid metabolism (Finkel et al. 2009).

Moreover, it has been observed that compounds such as folic acid, vitamin B-12, methionine, choline and betaine can affect DNA and histone methylation through altering the levels of SAM and S-adenosylhomocysteine, an inhibitor of methyltransferases (Mentch et al. 2015). Other water-soluble B vitamins (i.e. biotin, niacin, and pantothenic acid) play important roles in histone modifications (Kirkland 2009): biotin is a substrate for biotinylation, niacin is involved in ADP-ribosylation (as a substrate of poly(ADP-ribose) polymerase) and in acetylation (as a substrate of Sirt1) and pantothenic acid is a part of Coenzyme A to form acetyl-CoA, the metabolic intermediate required for histone acetylation. Ethanol significantly affects one-carbon metabolism by limiting methyltransferases reactions (Kaminen-ahola et al. 2010).

Furthermore, bioactive components of food can modify epigenetic phenomena through directly affecting enzymes involved in epigenetic mechanisms. For instance, genistein and tea catechin influence DNA methyltransferases (Dnmt) activity. Resveratrol, butyrate, sulforaphane, and diallyl sulfide inhibit HDAC and curcumin inhibits HAT (Nian et al. 2010; Chiu et al. 2009).

1.5 Transgenerational epigenetic inheritance

It has been suggested that a memory of former metabolic states exists and that transient changes in nutrition may have a long-lasting impact on gene expression (Vickers 2014), from previous generations and in particular from both mother and father (Woo and Patti 2008; Gluckman 2008; Ferguson-Smith and Patti 2011). The relevant aspect of paternal transmission is that sperm transmits only genetic and epigenetic factors, modeled by paternal diet, and that these spermal epigenetic perturbations transmitted to offspring influence adult metabolic phenotype.

Given all these evidences, it is conceivable to envision that focused modulation of epigenetic mechanisms through diet or specific nutrients may prevent diseases and maintain healthy status.

In this review, we will focus on the role of epigenetics in the regulation of lipid metabolism in rodent models and in humans, highlighting the importance of epigenetic remodeling in the pathophysiology of metabolic disorders.

2. Epigenetic regulation of lipid metabolism in rodents

2.1 DNA methylation

There are cumulating evidences that DNA methylation is susceptible to changes in environmental factors (Haan, Gevers, and Parker 1986; Henry et al. 1996; Staiger et al. 2006; Pogribny et al. 2006; Ghoshal et al. 2008). For example, the presence of hypermethylated CpG sites has been demonstrated upstream to TSS (transcription start site) of *Pparg* gene, the master regulator of adipogenesis, in visceral white adipose tissue (WAT) in response to high fat diet in mice (Fujiki et al. 2009).

The genome wide study of Multhaup et al. showed 232 differentially methylated regions (DMRs) of the DNA correlated with diet in murine adipocytes (Multhaup et al. 2015). This study highlighted hypermethylated regions near genes involved in lipid metabolic pathways, whereas hypomethylated regions correlated with inflammatory/immune pathways. For example, in adipocytes from high-fat diet fed mice they found hypermethylation sites within the promoter of Pck1 gene encoding Phosphoenolpyruvate Carboxykinase, which catalyses the rate-limiting step in glyceroneogenesis in adipose tissue, a key pathway for fatty acids storage as triacylglycerols. Moreover, this study reported for the first time DMRs in Fasn gene encoding fatty acid synthase; these DRMs correlated with differences in terms of glucose tolerance test. Accordingly, there are several evidences indicating that *de novo* lipogenesis in adipose tissue is impaired during metabolic dysfunction and that restoring this pathway selectively in WAT could be a strategy to improve obesity-dependent insulin resistance (Cao et al. 2008; Roberts et al. 2009; Huo et al. 2012). Of note, these authors also

found that DMRs are conserved across species. In particular, the authors identified 625 genome-wide DMRs in murine adipocytes that correlated with diet-induced obesity. Among these, 249 DMRs presented conserved methylation changes in human obesity and 170 had the same direction of methylation changes in both species. Moreover, 30 of these DMRs overlapped with SNPs associated with human T2D genetic risk.

Diet can also influence the transgenerational epigenetic inheritance, i.e. the transmission of phenotypic traits to the offspring via epigenetic modification in the germline (Daxinger and Whitelaw 2012) (Figure 2). Changes in DNA methylation level in the germline play a role in this mechanism. It has been clearly demonstrated that malnutrition during fetal life alters DNA methylation of specific genes and, as a consequence, increases the risk of developing obesity, insulin resistance in the following generation (Burdge et al. 2007). Recently, Martinez et al. (Martìnez et al. 2014) demonstrated that *in utero* undernutrition influences the lipogenic program in the liver of the second generation offspring, by reducing the expression of genes such as ATP-citrate lyase (*Acly*), Fatty acid synthase (*Fasn*), Steroyl CoA desaturase 1 (*Scd1*), Elongase of very long chain fatty acid 6 (*Elovl6*) and Sterol-response element binding factor-1 (*Srebf-1*). This dysregulation is due, at least in part, to reduced expression of Liver x receptor α (*Lxr-a or Nr1h3*), the master regulator of the lipogenic program in the liver, which results from reduced DNA methylation level at CpG 5' UTR promoter region and enrichment of repressive histone marks H3K9me2 and H3K27me3. This epigenetic signature was already present in sperm samples from their progenitors, meaning that it was inherited by somatic cells of offspring determining an altered metabolic phenotype. These mice showed moderated hypertriglyceridemia, increased hepatic cholesterol, increased VLDL production and reduced HDL cholesterol. Of note, this study highlighted how the peculiar combination of different epigenetic marks in a specific genetic locus determines the final molecular output in terms of gene expression.

Methylation of both DNA and histones is catalyzed by enzymes that use intermediates and cofactors belonging to one-carbon metabolism: methionine, folate, choline and vitamin B12 are important

precursors of these pathways. It is well known that deficiency of these nutrients may lead to hepatic fatty acid accumulation (Zivkovic et al. 2009; Kajikawa et al. 2011). At this regard, it has been shown that supplementation with dietary methyl donors in high fat sucrose (HFS) fed Wistar rats reduced hepatic fat accumulation with decreased level of global DNA methylation in supplemented control fed animals (Cordero et al. 2013). In particular, the authors showed changes in DNA methylation profile at CpG sites of Sterol Regulatory Element Biding Protein 2 (Srebf2), 1-acylglycerol-3-phosphate O-acyltransferase 3 (Agpat3), and Estrogen Receptor 1 (Esr1) promoters. Conversely, another study demonstrated that methionine restricted diets protects adult mice from age-related DNA hypomethylation in the liver. These investigators found increased hepatic global DNA methylation and reduced levels of S-adenosylhomocysteine (SAH), an important intermediate for DNA methylation reactions (Mattocks et al. 2016). These studies highlighted that diet may influence hepatic lipid metabolism by epigenetic control of gene expression. However, the opposite outcomes obtained in these studies highlights the need of more conclusive investigations to assess a possible link between methionine restriction and epigenetic regulation of lipid metabolism.

2.2 Histone modifications

Histone methylation is another epigenetic modification strictly linked with metabolism. It has been assessed that high fat diet (HFD) induces changes in chromatin methylation pattern, thus modulating the expression of genes involved in lipid metabolism. At this regards, Leung et al. (Leung et al. 2014) challenged C57BL/6J mice with HFD for 8 weeks and showed that HFD relaxed chromatin within *cis*-regulatory elements making them more accessible to transcription factors such as HNF4α (Hepatocyte Nuclear Factor 4α), FOXO1 (Fork-head box O1) and C/EBPα (CCAAT/enhancer binding protein α) in the liver. Moreover, they showed that chromatin sites, which upon HFD feeding become enriched in the activation histone mark H3K4me1 (monomethylated lysine 4 of histone H3), adopt an open conformation increasing their accessibility to transcription factors. Interestingly, these changes in chromatin accessibility were mirrored by

more than 300 genes differentially regulated in mice fed HFD, most of which implicated in lipid metabolism. In another study, Jun et al. (Jun et al. 2012) showed an altered H3K4me3 and H3K9me3 profile in primary hepatocytes treated with fatty acids, an *in vitro* model resembling early stage of hepatosteatosis. In particular, they found that the presence of these histone marks in the *Ppara* promoter paralleled the reduced expression of PPARα and of other genes involved in lipid catabolism. *Ppara* encodes Peroxisome Proliferator-activated receptor α, a nuclear receptor that activates the lipid catabolic program. This suggests that fatty acid treatment in primary hepatocytes triggers the alteration of histone methylation profile of *Ppara* promoter that reduces its expression and contributes to lipid accumulation and exacerbation of hepatosteatosis.

In 2009, two studies highlighted that the genetic ablation of H3K9-specific demethylase Jmjd1a, Jumonji domain containing 1A, induced metabolic impairment and obesity. Tateishi et al. (Tateishi et al. 2009) showed that mice lacking Jmjd1a developed obesity and hyperlipidemia in the adulthood. This was due to dysregulation of *Ppara* and *Ucp1*, two direct targets of Jmjd1a, in brown adipose tissue as a consequence of increased H3K9me2 levels at enhancers of these two genes. Moreover, they showed that *Jmjd1a* expression is induced upon β-adrenergic stimulation in brown adipose tissue (BAT). In addition, in the study of Inagaki et al. (Inagaki et al. 2009) the obese phenotype in Jmjd1a-/- mice correlated with reduced expression of several genes including those encoding the inhibitor of fat storage *ApoC1*, the insulin dependent glucose transporter *Glut4*, the anti-adipogenic transcription factor *CoupTFII* and *Adamts9*, which encodes a metalloprotease associated with type 2 diabetes. Recently, the same group disentangled the physiological role of Jmjd1a in the acute response to environmental factors, i.e. cold exposure, in brown adipose tissue. The authors demonstrated that Jmjd1a serves as a cAMP epigenetic sensor that induces a long-range looping of enhancer regions to transcription start site (TSS) to initiate transcription of thermogenic genes in BAT (Abe et al. 2015). In particular, they showed that Jmjd1a is phosphorylated at Ser265 by PKA in response to β-adrenergic activation. As a consequence, Jmjd1a forms a complex with Swi/SNF proteins and PPARγ upon enhancers of thermogenic genes Adrb1 and Ucp1, which brings

enhancers in proximity of TSS of these genes thus stabilizing the recruitment of RNA pol II and inducing their transcription. This mechanism was independent from the demethylase activity of the protein.

In a recent paper Mentch and colleagues (Mentch et al. 2015) showed that histone methylation dynamics senses rapid alterations in methionine metabolism. They found global alterations of histone methylation after methionine restriction *in vitro* and *in vivo*, with H3K4me3 levels being reduced within 2-4 hours after methionine deprivation. Since H3K4me3 marks active promoters, they evaluated also changes in gene transcription: one of the most down-regulated pathways was indeed a network of enzymes involved in one-carbon metabolism that showed reduced both H3K4me3 levels and mRNA levels. This suggested the inception of a feedback regulatory pathway to cope methionine restriction and maintain physiological homeostasis. Summarizing, this study demonstrated that cells could sense nutritional status and adapt to methionine restriction by modulating histone methylation levels and gene expression.

A mechanistic explanation whereby lipid metabolism may modulate both DNA and histone methylation remains to be fully addressed: possibly lipid metabolism may regulate the activity of enzymes involved in methylation/demethylation and/or may alter the level of intermediates/cofactors involved in these pathways. At this regard, Teperino et al. (Teperino, Schoonjans, and Auwerx 2010) hypothesized a link between intermediate metabolism and the activity of LSD1 (Lysine Specific Demethylase 1): LSD1 catalyzes demethylation reactions by using FAD, a cofactor in reactions of fatty acid β-oxidation pathway (i.e., acyl-CoA dehydrogenase).

A number of studies extensively demonstrated the tight link between histone deacetylases (HDACs) and metabolism. Several investigations dissected the role of specific histone deacetylases in the regulation of metabolism in specific tissues by using knock out murine models or chemical inhibitors (Chang et al. 2006; Potthoff et al. 2007; Knutson et al. 2008; Gao et al. 2009; Sun et al.

2011; Galmozzi et al. 2013). At this regard, Knutson et al. (Knutson et al. 2008) demonstrated that hepatic *Hdac3* deletion disrupted normal regulatory networks and altered completely metabolic homeostasis causing hepatomegaly, hepatosteatosis and impairments of glucose and lipid metabolism. These alterations were due to activation of PPARγ regulatory network within the liver. Indeed, HDACs regulatory activity depends upon the co-recruitment of multi-protein complexes. For example, HDAC3 deacetylase activity requires interaction with corepressors Silencing Mediator for Retinoid and Thyroid Receptors (SMRT) and Nuclear Receptor Corepressor (NCoR1)(Guenther, Barak, and Lazar 2001; Yu et al. 2003). Moreover, it has been demonstrated that HDAC3 interact with MeCP2, Methyl-CpG-binding Protein 2 (Kyle et al. 2016). In this work the authors showed that hepatic ablation of MeCP2 caused dyslipidemia and non-alcoholic fatty liver disease (NAFLD) as a result of altered expression of genes involved in lipogenesis pathways. MeCP2 deletion, in fact, disrupts HDAC3 recruitment on specific loci and increased H3K27ac marks at the Squalene epoxidase (*Sqle*), Fatty acid synthase (*Fasn*), and Cluster of differentiation 36 (*Cd36*) loci. The association of HDAC3 with hepatic genome is also regulated by the nuclear receptor Rev-erbα in a circadian fashion (Feng et al. 2011). During dark phase, when murine metabolism is active and in the feeding phase, low concentrations of Rev-erbα lead to dissociation of HDAC3 from the genome. Thus, acetylation levels at H3K9 are up-regulated during dark phase leading to increased expression in genes involved in hepatic *de novo* lipogenesis and this is reverted during light phase. Disruptions of this circadian mechanism lead to alteration in the epigenome that may contribute to the development of fatty liver.

HDAC3 plays an important role in the regulation of lipid metabolism also in the heart. At this regard, Montgomery and coworkers (Montgomery et al. 2008) reported that cardiac specific ablation of *Hdac3* upregulated expression of genes associated with an intense oxidative program (e.g., genes involved in fatty acid uptake, fatty acid oxidation, and electron transport chain/oxidative phosphorylation). These effects are related to hyperactivation of the nuclear receptor PPARα, which, together with HDAC3, forms a repressive complex on key target genes (*Ucp2*, *Ucp3*, *Pdk4*,

Acsl1, *Fatp*). However, Sun et al. (Sun et al. 2011) showed that *Hdac3* cardiac-ablated mice exposed to high fat diet developed hyperthrophic cardiomyopathy, heart failure and lethality. In this experimental setting *Hdac3* ablation was, in fact, accompanied by downregulation of genes involved in lipid catabolism and fatty acid oxidation, thus *Hdac3* heart-specific knock out mice were not able to metabolize efficiently the dietary lipids and died from heart failure. This study demonstrated that HDAC3 transcriptionally regulates lipid catabolism in the heart and that loss of HDAC3 reduces the ability to respond to lipid overload, highlighting that high fat feeding reverts metabolic remodeling observed upon *Hdac3* ablation in mice fed low fat diet (reported in Montgomery et al. 2008).

Acetyl-CoA is as an important substrate of histone acetyltransferase (HAT) enzymes and represents the source of acetyl groups for histone acetylation (Wellen et al. 2009), indicating a link between metabolism and epigenetics. The nuclear ATP-citrate lyase (ACLY), the enzyme that converts citrate into acetyl-CoA and oxaloacetate, provides acetyl-CoA required to sustain histone acetylation. In the same study, these authors also demonstrated that reduced glucose concentration affected ACLY-dependent histone acetylation, thus suggesting that glucose metabolism is the major source of acetyl groups for acetylating chromatin.

It would be interesting to verify whether, under particular metabolic conditions, lipid catabolism (i.e. fatty acid β-oxidation) could also be a source of acyl-CoA for chromatin acetylation.

2.3 Non-coding RNA

The discovery that non-coding RNAs (ncRNAs) account for almost two thirds of all transcripts raised interest in this fascinating mechanism of gene regulation. Some evidences support the possibility that long non-coding RNAs (lncRNAs) play a role in the regulation of lipid metabolism. At this regard, a liver-specific lncRNAs, named long non-coding liver-specific triglyceride regulator (lncSTR) (Li et al. 2015), whose expression is influenced by the nutritional status, has been identified. Knock down of lncSTR *in vivo* increased triglycerides clearance also in a setting

of hyperlipidaemia, the ApoE $^{-/-}$ mouse. The authors have also found that lncSTR is part of a molecular complex with TAR DNA binding protein 43 (TDP-43) that regulates the expression of Cytochrome P450 8b1 (Cyp8b1), a key enzyme in the bile acid synthesis pathway. This mechanism determines a specific bile acid pool that activates Farnesoid -X- receptor (FXR), which, in turn, induces apoC2 expression.

Recently, Yang and colleagues used an integrated approach to identify 359 putative long non coding RNAs (lncRNA) that function as metabolic regulators in liver, adipose tissue and skeletal muscle (Yang et al. 2016). They found Gm16551, a liver-specific lncRNA strongly regulated by the master transcription factor of lipogenesis, SREBF1c. Liver-specific Gm16551 knock down mice showed increased expression of lipogenic genes *Fasn*, *Acly* and *Scd1* and elevated circulating levels of triglycerides.

miRNA is the family of ncRNAs better characterized and many investigators assessed their importance in the regulation of lipid metabolism. *In vivo* inhibition of miR-122, the predominant miRNA in the liver (Lagos-Quintana et al. 2002), reduced plasma cholesterol levels, hepatic fatty acid and cholesterol synthesis and enhanced hepatic fatty acid β-oxidation in lean mice (Esau et al. 2006). miR-122 inhibition, in fact, decreased expression of genes involved in fatty acid synthesis such as *Fasn*, *Acc1*, *Acc2*, *Scd1*, and *Acly*. When fed high fat diet, mice treated with miR-122 inhibitor showed decreased cholesterolemia and ameliorated liver steatosis with reduced levels of hepatic triglycerides. These results identified miR-122 as a regulator of cholesterol and fatty acid metabolism in the liver, suggesting it as an attractive therapeutic target for metabolic disorders. In 2010 Rayner et al. (Rayner et al. 2010) discovered miR-33 in an intronic sequence of SREBF2 (Sterol Regulatory Element Binding factorr 2) gene, the master regulator of cholesterol homeostasis. This miRNA suppresses the expression of the cholesterol ATP-binding cassette A1 (ABCA1) and other genes involved in cholesterol transport, thus reducing plasma levels of HDL-cholesterol In a follow-up study the same group demonstrated that inhibition of miR-33 with antisense oligonucleotide reduced atherosclerotic plaques size in LDL-Receptor deficient mice, a

well-established model of atherosclerosis (Rayner et al. 2011). These oligonucleotides increased cholesterol efflux in the macrophages within the plaque, suggesting it as a promising strategy to treat cardiovascular disease.

Moreover, it has been demonstrated that miR-33 has a central role in the cross talk regulation of cholesterol and fatty acid metabolism. SREBF1c, the hepatic regulator of *de novo* fatty acid synthesis, is itself negatively regulated by miR-33 (Horie et al. 2013). mir-33$^{-/-}$ mice showed, in fact, increased body weight and hepatic steatosis due to increased expression of SREBF1 and its target genes, such as acetyl-CoA carboxylase (*Acaca*), fatty acid synthase (*Fasn*), Elongase of very long chain fatty acid 6 (*Elovl6*) and stearoyl-CoA desaturase (*Scd1*). These findings suggested that upregulation of miR-33 enhances cholesterol production in sterol depleted conditions. On the contrary, when cholesterol homeostasis is reached, decreased expression of miR-33 derepresses *Srebf1* expression and consequently fatty acid synthesis is favoured. Another miRNA involved in the regulation of lipid metabolism is miR-335. miR-335 is up regulated in liver and white adipose tissue in different murine models of obesity including *ob*/*ob*, *db*/*db*, and KKAy mice (Nakanishi et al. 2009).

Recently, it has been demonstrated that miRNAs could act in a concerted fashion to regulate lipid metabolism (Jeon et al. 2013). For example, mir-182 and miR-96 are expressed from the same polycistronic locus in response to transcription factor SREBP-2. By acting as a "miRNA operon" this two miRNAs reduce the expression of F-box and WD-40 domain protein 7 (Fbxw7), and Insig-2, insulin induced gene 2, respectively, two genes that negatively regulate SREBP2 by reducing its nuclear localization.

3. Epigenetics of lipid metabolism in humans

3.1 EWAS studies and DNA methylation

Animal studies provided important evidences about the role of epigenetics in the regulation of lipid metabolism and in the onset of metabolic diseases, paving the way for in depth analysis about the role of epigenetics in human metabolism. In the last few years, new multi-dimensional approaches have been used to understand how genetics and epigenetics interplay in pathophysiology of metabolic disorders. Giving the worrisome increase of obesity in western world, several studies focused their attention on epigenetics of metabolic alterations underlying obesity and overweight. It was demonstrated that the obesity risk allele FTO, encoding for Fat mass and obesity-associated protein also known as α-ketoglutarate-dependent dioxygenase, is associated with higher methylation of sites within intron one of the FTO gene, caused by CpG site creating SNPs (Bell et al. 2010). The authors described an interaction between genetic and epigenetic factors for the risk allele. More recently, a genome wide analysis on peripheral whole blood determined five sites, corresponding to six genes (*KARS, TERF2IP, DEXI, MSI1, STON1* and *BCAS3*) that differ in methylation level between homozygous carriers of the normal and the risk allele of the FTO gene and twenty sites that correlate with obesity (Almén et al. 2012). In the last few years, deep investigations of epigenome in human populations have been performed and epigenome-wide association studies (EWAS) became a powerful instrument to investigate how profoundly dietary habits and lifestyle can contribute to epigenetic changes and how this rearrangement correlates with metabolic dysfunctions. Very large EWAS including 5465 subjects investigated the association of adiposity, defined by body mass index and waist circumference, and DNA methylation at numerous CpG sites in blood cells (Demerath et al. 2015). This study highlighted that several biological pathways are significantly enriched for methylation in association with obesity, including those involved in lipid and energy metabolism. Among the specific loci differentially methylated the authors reported carnitine palmitoyl transferase 1a (*CPT1A*, involved in mitochondrial uptake of long-chain fatty acids and triglyceride metabolism), ATP Binding Cassette Subfamily G Member 1 (*ABCG1*, involved in macrophage cholesterol and phospholipids transport, and lipid homeostasis), and Sterol Regulatory Element Binding Transcription Factor 1 (*SREBF1*, a key regulator of

lipogenesis). Another large-scale genome-wide analysis of the association between adult body mass index (BMI) and DNA methylation identified a correlation between BMI and methylation of a component of the hypoxia inducible transcription factor HIF3A, in blood and adipose tissue (Dick et al. 2014). This result is in line with other reports demonstrating that HIF system is involved in metabolic regulation and energy expenditure (Jiang et al. 2011). Recently, an association between adipose tissue DNA methylation and mRNA expression in human adipose tissue and three risk factors for metabolic diseases, i.e. age, BMI and HbA1c (a measure of long-term glycaemia) has also been shown (Rönn et al. 2015). The authors found significant correlations between age and methylation in CpG sites in genes involved in lipid metabolism, such as fatty acid elongase 2 (*ELOVL2*) in both blood and adipose cells. Of note, this evidence highlights that blood-based epigenetic biomarkers mirror epigenetic signatures in metabolic tissues, suggesting that epigenetic marks may become interesting biomarkers of metabolic disorders in obesity, T2D and related diseases. The possibility of a rapid identification of these epigenetic biomarkers could allow a prompt and appropriate therapeutic intervention. Another important gene whose DNA methylation was increased in human adipose tissue and blood cells with increased age was Kruppel-like factor 14 (*KFL14*). GWAS studies identified an association between SNPs near KFL14 and type 2 diabetes and HDL cholesterol levels(Wang et al. 2014), further confirming the strict link between genetics and epigenetics in metabolic regulation, and providing new insights in the pathophysiology of obesity and related disorders. At this regard, Ahrens and co-workers (Ahrens et al. 2013) reported that non-alcoholic fatty liver disease (NAFLD), a pathologic condition associated to obesity, correlates with a peculiar hepatic methylation signature. The authors found NAFLD-specific expression and methylation differences for genes regulating metabolism. Among these genes the authors identified ATP citrate lyase (*ACLY*). ACLY, catalysing the conversion of citrate into acetyl-CoA, is the first enzyme of fatty acid biosynthesis(Linn and Srere 1979) Non-alcoholic steatohepatitis determined decreased DNA methylation of *ACLY* gene, paralleled by higher *ACLY* mRNA expression, indicating activation of a lipogenic pathway in NAFLD patients. Moreover, the

authors compared liver biopsies before and after bariatric surgery and found that NAFLD-associated methylation changes are partially reversible, demonstrating the possibility to remodel human epigenome through a therapeutic intervention. However, the possibility to selectively manipulate DNA methylation at specific epigenomic regions is a really challenging task, rather we envision the value of identifying differences in DNA methylation as useful biomarkers for the efficacy of pharmacological approaches in metabolic disorders.

Another organ whose functionality is impaired in obesity is skeletal muscle. In skeletal muscle of obese patients the rate of fatty acid β-oxidation (FAO) is reduced (Kim et al. 2000), thus contributing to ectopic lipid accumulation. Maples et al (Maples et al. 2015) recently reported that blunted expression of carnitine palmitoyltransferase-1B (CPT1B) in primary human skeletal muscle cultures from obese women was accompanied by changes in CpG methylation. CPT1B mediates the transfer of long-chain fatty acids from the cytosol to the mitochondrial matrix and is a rate-limiting step in FAO. The authors found that, in response to a lipid overload, cell cultures from lean subjects showed increased expression of CPT1B, as result of reduced cytosine methylation in CPT1B promoter. In cells from obese patients this epigenetic remodelling is blunted, preventing the binding of key transcriptional activators such as peroxisome proliferator-activated receptor-δ (PPARδ) and hepatocyte nuclear factor 4α (HNF4α) to CPT1B promoter.

Recently, an epigenome-wide association showed a correlation between obesity and deep changes in DNA methylation (Wahl et al. 2017) . The authors demonstrated these modifications occur as result of adiposity and they are not to

be considered as the cause of obesity and related metabolic impairment. Interestingly from this study emerged the existence of sentinel methylation markers, associated to obesity, that determine gene expression signatures at specific loci, mostly corresponding to genes involved in lipid and lipoprotein metabolism, substrate transport and inflammatory pathways. The authors also demonstrated that alterations of DNA methylation profile on these loci could be predictive of the

future development of obesity-related disease, such as type 2 diabetes, paving the way for new prevention strategy for the onset of this pathology.

All these experimental evidences demonstrate that DNA methylation profile strongly influences lipid metabolic pathways in several organs participating in energy homeostasis, and link indissolubly metabolic disorders and epigenome remodelling.

It is important to notice that prenatal environment could influence DNA methylation. A study on Gambian pregnant women demonstrate that seasonal alterations in methyl-donor nutrient intake at the time of conception influences several plasma biomarkers predicting differential DNA methylation in lymphocytes and hair follicles obtained from infants postnatally(Dominguez-salas et al. 2014). Other authors investigated genome-wide DNA methylation on whole blood of adult offspring whose mothers were exposed to famine during the Dutch Hunger Winter (Heijmans et al. 2008). This study highlighted that differential DNA methylation occurred only in subjects that were exposed to famine during early gestation (periconceptional exposure). Among the DNA regions differentially methylated, the authors identified genomic portion of INSR and CPT1A that were shown to have enhancer activity *in vitro*: differential methylation in these sites has been associated with birth weight and serum LDL-cholesterol. These results prove the link between the timing of nutritional challenges and epigenetic remodelling, which can alter metabolism.

3.2 Histone modifications

In analogy to evidences coming from studies in rodents, a link between metabolic disorders and histone modifications has also been demonstrated in humans. It has in fact been shown that global histone H3 methylation status changes in response to overweight: in particular the authors reported decreased lysine 4 dimethylation of histone H3 (H3K4me2) in adipocytes from diabetic and non diabetic obese subjects (Jufvas et al. 2013). On the other hand, they found that trimethylation of H3K4 (H3K4me3) was 40% higher in adipocytes from overweight diabetic subjects compared with normal-weight and overweight non-diabetic subjects. These results highlight that obesity and

diabetes correlates with altered histone methylation profile in adipocytes. Further epigenome wide studies with ChIP-seq are required to identify the differences in histone methylation in selective regions, to understand how histone methylation/demethylation is able to reprogram adipose tissue metabolism, identifying potential new regulatory circuits that could be exploited for the treatment of these disorders.

Remarkably, histone modifications at very early stages of differentiation determine long term rearrangement of metabolic signalling pathways. At this regard, Joseph and coworkers (Joseph et al. 2016) mapped chromatin state for histone marks H3K27 acetylation (a mark of active chromatin) and H3K27 trimethylation (a repressing mark) in adipocytes obtained by differentiating mesenchymal stem cells (MSCs) from umbilical cord tissue of individuals who were born small for gestational age (SGA) or of normal neonates. Interestingly, it is known that SGA subjects have higher risk to develop metabolic diseases in the adulthood (Varvarigou 2010). Analysing these epigenetic modifications the authors were able to demonstrate that acyl-coenzyme A synthetase 1 (ACSL1), which converts free long-chain fatty acids into fatty acyl-CoA esters (Soupene and Kuypers 2008), is associated with increased histone H3K27ac in adipocytes derived from MSCs obtained from SGA children. This evidence further demonstrated the existence of strict link between epigenetics and regulation of lipid metabolism in humans. Moreover, these results highlight that histones modifications occurring in cellular precursors could be maintained along differentiation phases, even when cells are cultured *in vitro*.

Recent evidences demonstrated that histone modifications are important determinants also of hepatic metabolic signature: it has been shown that lysine-specific demethylase 2 (LSD2) regulates lipid metabolism in human hepatocytes (Nagaoka et al. 2015). The authors reported that LSD2, demethylating H3K4, is able to repress genes involved in lipid influx and metabolism, and that c-Jun mediates recruitment of LSD2 on these genes. Interestingly they demonstrated that knock down of LSD2 increased intracellular levels of many lipid metabolites, thus determining higher susceptibility to cellular damage induced by exposure to free fatty acids.

3.3 microRNAs

In recent years we have witnessed a growing interest in microRNAs in the pathophysiology of nonalcoholic steatohepatitis (NASH). Cheung and coworkers (Cheung et al. 2008), by comparing the expression of 474 human microRNAs in subjects with NASH versus controls (without lipid deposition in the liver), found that the expression of miR-122, the most abundant miRNA in the liver, was reduced in NASH. As a consequence of miR122 underexpression, the authors reported increased protein levels of its targets fatty acid synthase (FASN), 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase) and their transcriptional activators, the sterol-response-element-binding protein-1c and 2 (SREBP-1c, SREBP-2), which are involved in lipogenic pathways. This determines the ectopic lipid accumulation resulting in NASH. More recently, other authors identified 42 novel miRNAs differentially regulated in NAFLD as compared to non-NAFLD liver (Soronen et al. 2016). PPARα/RXRα activation pathway was identified among the target pathways of these miRNAs. Indeed PPARα activates β-oxidation and it is known that its expression is reduced in NAFLD (Tyagi et al. 2011). Suppression of the PPARα/RXRα pathway by miRNAs reduces the rate of fatty acid oxidation, thus favouring lipogenesis and progression of liver disease.

4. Conclusions

The evidences from human and animal studies here reviewed (Figure 3) demonstrated that nutritional and lifestyle factors (both pre- and postnatal) impact epigenome remodelling, and finely regulate lipid metabolism. The study of epigenetic modifications related to metabolic pathologies in rodents is a valuable strategy in order to identify new targets for the treatment of human diseases. At this regard a similar DNA methylation pattern in murine and human obesity has been shown: to date 249 regions differentially methylated in animal and human studies have been identified, and 30

of these regions overlapped with SNPs associated to genetic risk to develop type 2 diabetes in human patients. Despite this overlapping, rodents and humans also showed different mechanism of regulation of lipid metabolism, since obesity in mice remodel DNA methylation upstream of *Pparg* gene and on *Pck1* promoter, thus affecting glyceroneogenesis lipid and accumulation in WAT. Similar alterations have not been described in human WAT. On the other hand, in human adipocytes it has been proved a correlation between age and methylation at CpG sites in another gene playing key role in lipid metabolism, ELOVL2. Also, liver metabolism showed epigenetic regulation of some key actors in both mice and humans, even though this does not always occur through the same mechanisms; among these factors particularly relevant is the role of the lipogenic enzyme ATP citrate lyase (ACLY). The expression of this lipogenic gene in mouse liver is reduced upon maternal undernutrition, as a consequence of epigenetic switch off of *Lxra* gene. On the contrary, ACLY expression is induced through a reduction of DNA methylation status in human NASH. These evidences reveal a fine-tuning epigenetic regulation of ACLY, fundamental to maintain the balance of hepatic lipid metabolism. It is intriguing to notice that also other epigenetic mechanisms were conserved among species in the maintenance of lipid metabolism in liver. Among those a role is played by miR122, that was found to regulate lipid and cholesterol metabolism in murine models, but also resulted reduced in human NASH, arising as important modulator of lipid metabolism in human liver.

This review highlighted that an important link between epigenetic regulation and metabolic health outcomes has been recognized, determining the upsurge of new interest in this field. The possibility to widely study epigenetic modifications through ChIP sequencing, together with the reduced cost of epigenome-wide association studies could allow bridging the observations coming from murine models in the context of human diseases. These investigations will shed new light in the knowledge of the strict link between epigenome, environment and metabolic pathologies, paving the way for new diagnosis and identification of novel potential therapeutic interventions.

Acknowledgements

We would like to thank other members of the laboratory for valuable discussion and Elda Desiderio Pinto for administrative support. Supported by EU FP7 HUMAN 602757, FP7 NR-NET PITN-GA-2013-606806 and CARIPLO Foundation 2015-0641 to MC.

Figure captions

Figure 1

Epigenetic mechanisms regulating genome function. (A) Environment influences chromatin state. Nutrients, life style and environmental stressors act as modulators of the epigenetic machinery, thus regulating the accessibility of DNA to transcription factors and RNA polymerase II, and establishing peculiar patterns of gene expression that are the results of a balance between activating and inhibiting DNA and histone modifications. (B) Furthermore, non-coding RNAs (*ncRNAs*), such as microRNAs (*miRNA* - 18-25 nt) and long non-coding RNAs (*lncRNAs* > 200nt), influence gene expression at the level of transcription or translation through the interaction with DNA and mRNA, and play a role in chromatin regulation by interacting with chromatin remodeling enzymes or transcription factors.

Figure 2

Transgenerational epigenetic inheritance. Environmental stimuli, such as lifestyle, exercise, smoke, etc. determine specific epigenomic patterns in the germline of an adult subject that could be transmitted to the offspring. This results in specific phenotypic traits such as predisposition to diseases that affect individuals in their entire life.

Figure 3

Summary of the main epigenetic modifications described in the text correlated to environmental changes in mouse models and humans.

References

Abe, Yohei, Royhan Rozqie, Yoshihiro Matsumura, Takeshi Kawamura, Ryo Nakaki, Yuya Tsurutani, Kyoko Tanimura-inagaki, et al. 2015. "JMJD1A Is a Signal-Sensing Scaffold That Regulates Acute Chromatin Dynamics via SWI/SNF Association for Thermogenesis." *Nature Communications* 6 (May). Nature Publishing Group: 1–14. doi:10.1038/ncomms8052.

Ahrens, Markus, Ole Ammerpohl, Witigo Von Schonfels, Julia Kolarova, Susanne Bens, Timo Itzel, Andreas Teufel, et al. 2013. "Short Article DNA Methylation Analysis in Nonalcoholic Fatty Liver Disease Suggests Distinct Disease-Specific and Remodeling Signatures after Bariatric Surgery." *Cell Metabolism* 18: 296–302. doi:10.1016/j.cmet.2013.07.004.

Almén, Markus Sällman, Josefin A. Jacobsson, George Moschonis, Christian Benedict, George P Chrousos, Robert Fredriksson, and Helgi B Schiöth. 2012. "Genomics Genome Wide Analysis Reveals Association of a FTO Gene Variant with Epigenetic Changes." *Genomics* 99: 132–37. doi:10.1016/j.ygeno.2011.12.007.

Barth, Teresa K., and Axel Imhof. 2010. "Fast Signals and Slow Marks: The Dynamics of Histone Modifications." *Trends in Biochemical Sciences* 35 (11). Elsevier Ltd: 618–26. doi:10.1016/j.tibs.2010.05.006.

Bell, Christopher G, Sarah Finer, Cecilia M Lindgren, Gareth A Wilson, Vardhman K Rakyan, Andrew E Teschendorff, Pelin Akan, et al. 2010. "Integrated Genetic and Epigenetic Analysis Identifies Haplotype-Specific Methylation in the FTO Type 2 Diabetes and Obesity Susceptibility Locus." *PLoS ONE* 5 (11). doi:10.1371/journal.pone.0014040.

Burdge, Graham C, Jo Slater-jefferies, Christopher Torrens, Emma S Phillips, Mark A Hanson, and Karen A Lillycrop. 2007. "Dietary Protein Restriction of Pregnant Rats in the F0 Generation Induces Altered Methylation of Hepatic Gene Promoters in the Adult Male Offspring in the F1 and F2 Generations." *British Journal of Nutrition* 97: 435–39. doi:10.1017/S0007114507352392.

Cao, Haiming, Kristin Gerhold, Jared R Mayers, Michelle M Wiest, and Steven M Watkins. 2008. "Identification of a Lipokine, a Lipid Hormone Linking Adipose Tissue to Systemic Metabolism." *Cell* 134: 933–44. doi:10.1016/j.cell.2008.07.048.

Chang, Shurong, Bryan D Young, Shijie Li, Xiaoxia Qi, James A Richardson, and Eric N Olson. 2006. "Histone Deacetylase 7 Maintains Vascular Integrity by Repressing Matrix Metalloproteinase 10." *Cell* 126: 321–34. doi:10.1016/j.cell.2006.05.040.

Cheung, Onpan, Puneet Puri, Faridoddin Mirshahi, James W Maher, John M Kellum, Haeki Min, D Ph, Velimir A Luketic, and Arun J Sanyal. 2008. "Nonalcoholic Steatohepatitis Is Associated With Altered Hepatic Micro RNA Expression." *Hepatology* 48 (6): 1810–20. doi:10.1002/hep.22569.NONALCOHOLIC.

Chiu, Jane, M Sc, Zia A Khan, D Ph, Hana Farhangkhoee, M Sc, Subrata Chakrabarti, and D Ph. 2009. "Curcumin Prevents Diabetes-Associated Abnormalities in the Kidneys by Inhibiting p300 and Nuclear Factor-kB." *Nutrition Journal* 25: 964–72. doi:10.1016/j.nut.2008.12.007.

Choi, Sang-woon, and Simonetta Friso. 2010. "Epigenetics : A New Bridge between Nutrition and Health." *Advances in Nutrition* 1: 8–16. doi:10.3945/an.110.1004.8.

Cordero, Paul, Javier Campion, Fermin I Milagro, and J Alfredo Martinez. 2013. "Transcriptomic and Epigenetic Changes in Early Liver Steatosis Associated to Obesity: Effect of Dietary Methyl Donor Supplementation." *Molecular Genetics and Metabolism* 110 (3). United States: 388–95. doi:10.1016/j.ymgme.2013.08.022.

Daxinger, Lucia, and Emma Whitelaw. 2012. "Understanding Transgenerational Epigenetic Inheritance via the Gametes in Mammals." *Nature Reviews* 13 (3). Nature Publishing Group: 153–62. doi:10.1038/nrg3188.

Demerath, Ellen W, Weihua Guan, Megan L Grove, Stella Aslibekyan, Michael Mendelson, Yi-hui Zhou, Åsa K Hedman, et al. 2015. "Epigenome-Wide Association Study (EWAS) of BMI , BMI Change and Waist Circumference in African American Adults Identi Fi Es Multiple Replicated Loci." *Human Molecular Genetics* 24 (15): 4464–79. doi:10.1093/hmg/ddv161.

Dick, Katherine J, Christopher P Nelson, Loukia Tsaprouni, Johanna K Sandling, Dylan Aïssi, Simone Wahl, Eshwar Meduri, et al. 2014. "DNA Methylation and Body-Mass Index : A Genome-Wide Analysis." *The Lancet* 383: 1990–98. doi:10.1016/S0140-6736(13)62674-4.

Dominguez-salas, Paula, Sophie E Moore, Maria S Baker, Andrew W Bergen, Sharon E Cox, Roger A Dyer, Anthony J Fulford, et al. 2014. "Maternal Nutrition at Conception Modulates DNA Methylation of Human Metastable Epialleles." *Nature Communications* 5. Nature Publishing Group: 1–7. doi:10.1038/ncomms4746.

Esau, Christine, Scott Davis, Susan F Murray, Xing Xian Yu, Sanjay K Pandey, Michael Pear, Lynnetta Watts, et al. 2006. "miR-122 Regulation of Lipid Metabolism Revealed by in Vivo Antisense Targeting." *Cell Metabolism* 3 (February): 87–98. doi:10.1016/j.cmet.2006.01.005.

Feng, Dan, Tao Liu, Zheng Sun, Anne Bugge, Shannon E Mullican, Theresa Alenghat, X. Shirley Liu, and Mitchell A. Lazar. 2011. "A Circadian Rhythm Orchestrated by Histone Deacetylase 3 Controls Hepatic Lipid Metabolism." *Science* 331: 1315–20. doi:10.1126/science.1198125.

Ferguson-Smith, Anne C., and Mary Elizabeth Patti. 2011. "You Are What Your Dad Ate." *Cell Metabolism* 13 (2). Elsevier Inc.: 115–17. doi:10.1016/j.cmet.2011.01.011.

Ferrari, Alessandra, Erika Fiorino, Marco Giudici, Federica Gilardi, Andrea Galmozzi, Nico Mitro, Gaia Cermenati, et al. 2012. "Linking Epigenetics to Lipid Metabolism: Focus on Histone

Deacetylases." *Molecular Membrane Biology* 29 (7): 257–66. doi:10.3109/09687688.2012.729094.

Finkel, Toren, Chu-Xia Deng, and Raul Mostoslavsky. 2009. "Recent Progress in the Biology and Physiology of Sirtuins." *Nature* 460 (7255): 587–91. doi:10.1038/nature08197.Recent.

Fujiki, Katsunori, Fumi Kano, Kunio Shiota, and Masayuki Murata. 2009. "Expression of the Peroxisome Proliferator Activated Receptor γ Gene Is Repressed by DNA Methylation in Visceral Adipose Tissue of Mouse Models of Diabetes." *BMC Biology* 7: 1–14. doi:10.1186/1741-7007-7-38.

Galmozzi, Andrea, Nico Mitro, Alessandra Ferrari, Elise Gers, Federica Gilardi, Cristina Godio, Gaia Cermenati, et al. 2013. "Inhibition of Class I Histone Deacetylases Unveils." *Diabetes* 62 (March): 732–42. doi:10.2337/db12-0548.

Gao, Zhanguo, Jun Yin, Jin Zhang, Robert E Ward, Roy J Martin, Michael Lefevre, William T Cefalu, and Jianping Ye. 2009. "Butyrate Improves Insulin Sensitivity and Increases Energy Expenditure in Mice." *Diabetes* 58 (7): 1509–17. doi:10.2337/db08-1637.

Ghoshal, Kalpana, Xin Li, Jharna Datta, Shoumei Bai, Igor Pogribny, and Marta Pogribny. 2008. "A Folate- and Methyl-Deficient Diet Alters the Expression of DNA Methyltransferases and Methyl CpG Binding Proteins Involved in Epigenetic Gene Silencing in Livers of F344 Rats." *Journal of Nutrition* 136 (6): 1522–27.

Gluckman, Peter D. 2008. "Effect of In Utero and Early Life Conditions on Adult Health and Disease." *The New England Journal of Medicine* 359 (1): 61–73. doi:10.1056/NEJMra0708473.Effect.

Guenther, Matthew G, O R R Barak, and Mitchell A Lazar. 2001. "The SMRT and N-CoR Corepressors Are Activating Cofactors for Histone Deacetylase 3." *Molecular and Cellular Biology* 21 (18): 6091–6101. doi:10.1128/MCB.21.18.6091.

Haan, Judy B De, Wieland Gevers, and M Iqbal Parker. 1986. "Effects of Sodium Butyrate on the Synthesis and Methylation of DNA in Normal Cells and Their Transformed Counterparts1." *Cancer Research* 46 (February): 713–16.

Heijmans, Bastiaan T, Elmar W Tobi, Aryeh D Stein, Hein Putter, Gerard J Blauw, Ezra S Susser, P Eline Slagboom, and L H Lumey. 2008. "Persistent Epigenetic Differences Associated with Prenatal Exposure to Famine in Humans." *Proceedings of the National Academy of Sciences of the United States of America* 105 (44). United States: 17046–49. doi:10.1073/pnas.0806560105.

Henry, Robert R, Theodore P Ciaraldi, Leslie Abrams-carter, Sunder Mudaliar, Kyong Soo Park, and Svetlana E Nikoulina. 1996. "Glycogen Synthase Activity Is Reduced in Cultured Skeletal Muscle Cells of Non – Insulin-Dependent Diabetes Mellitus Subjects Biochemical and Molecular Mechanisms." *The Journal of Clinical Investigation* 98 (5): 1231–36.

Horie, Takahiro, Tomohiro Nishino, Osamu Baba, Yasuhide Kuwabara, Tetsushi Nakao, Masataka Nishiga, Shunsuke Usami, et al. 2013. "MicroRNA-33 Regulates Sterol Regulatory Element-Binding Protein 1 Expression in Mice." *Nature Communications* 4. Nature Publishing Group: 1–12. doi:10.1038/ncomms3883.

Hulsmans, Maarten, Dieuwke De Keyzer, and Paul Holvoet. 2016. "MicroRNAs Regulating Oxidative Stress and Inflammation in Relation to Obesity and Atherosclerosis." *The FASEB Journal* 25 (8): 2515–27. doi:10.1096/fj.11-181149.

Huo, Yuqing, Xin Guo, Honggui Li, Hang Xu, Vera Halim, Weiyu Zhang, Huan Wang, et al. 2012. "Targeted Overexpression of Inducible 6-Phosphofructo-2-Kinase in Adipose Tissue Increases Fat Deposition but Protects against Diet-Induced Insulin Resistance and Inflammatory Responses *." *The Journal of Biological Chemistry* 287 (25): 21492–500. doi:10.1074/jbc.M112.370379.

Inagaki, Takeshi, Makoto Tachibana, Kenta Magoori, Hiromi Kudo, Toshiya Tanaka, Masashi Okamura, Makoto Naito, and Tatsuhiko Kodama. 2009. "Obesity and Metabolic Syndrome in Histone Demethylase JHDM2a-Deficient Mice." *Genes to Cells* 14: 991–1001. doi:10.1111/j.1365-2443.2009.01326.x.

Jang, Hyeran, and Carlo Serra. 2014. "Nutrition, Epigenetics, and Diseases." *Clinical Nutrition Research* 3: 1–8. doi:10.7762/cnr.2014.3.1.1.

Jeon, Tae-il, Ryan M Esquejo, Manuel Roqueta-rivera, Peter E Phelan, Young-ah Moon, Subramaniam S Govindarajan, Christine C Esau, and Timothy F Osborne. 2013. "An SREBP-Responsive microRNA Operon Contributes to a Regulatory Loop for Intracellular Lipid Homeostasis." *Cell Metabolism* 18 (1). Elsevier Inc.: 51–61. doi:10.1016/j.cmet.2013.06.010.

Jiang, Changtao, Aijuan Qu, Tsutomu Matsubara, Tatyana Chanturiya, William Jou, Oksana Gavrilova, Yatrik M Shah, and Frank J Gonzalez. 2011. "Disruption of Hypoxia-Inducible Factor 1 in Adipocytes Improves Insulin Sensitivity and Decreases Adiposity in High-Fat Diet – Fed Mice." *Diabetes* 60 (11): 2484–95. doi:10.2337/db11-0174.

Joseph, Roy, Jeremie Poschmann, Rami Sukarieh, Peh Gek Too, Sofi G Julien, Feng Xu, Ai Ling Teh, et al. 2016. "ACSL1 Is Associated With Fetal Programming of Insulin Sensitivity and Cellular Lipid Content Oil Red O Staining." *Molecular Endocrinology* 29 (June 2015): 909–20. doi:10.1210/me.2015-1020.

Jufvas, Åsa, Simon Sjödin, Kim Lundqvist, Risul Amin, Alexander V Vener, and Peter Strålfors. 2013. "Global Differences in Specific Histone H3 Methylation Are Associated with Overweight and Type 2 Diabetes." *Clinical Epigenetics* 5 (15): 2–7. doi:10.1186/1868-7083-5-15.

Jun, Hee-jin, Jinyoung Kim, Minh-hien Hoang, and Sung-joon Lee. 2012. "Hepatic Lipid Accumulation Alters Global Histone H3 Lysine 9 and 4 Trimethylation in the Peroxisome

Proliferator-Activated Receptor Alpha Network." *PLoS ONE* 7 (9): 1–8. doi:10.1371/journal.pone.0044345.

Kajikawa, Satoshi, Kazunori Imada, Takashi Takeuchi, Yutaka Shimizu, Akiko Kawashima, Tsuyoshi Harada, and Kiyoshi Mizuguchi. 2011. "Eicosapentaenoic Acid Attenuates Progression of Hepatic Fibrosis with Inhibition of Reactive Oxygen Species Production in Rats Fed Methionine- and Choline-Deficient Diet." *Digestive Diseases and Sciences* 56 (4). United States: 1065–74. doi:10.1007/s10620-010-1400-5.

Kaminen-ahola, Nina, Arttu Ahola, Murat Maga, Kylie-ann Mallitt, Paul Fahey, Timothy C Cox, Emma Whitelaw, and Suyinn Chong. 2010. "Maternal Ethanol Consumption Alters the Epigenotype and the Phenotype of Offspring in a Mouse Model." *PLoS Genetics* 6 (1): e1000811. doi:10.1371/journal.pgen.1000811.

Katada, Sayako, Axel Imhof, and Paolo Sassone-Corsi. 2012. "Connecting Threads: Epigenetics and Metabolism." *Cell* 148 (1–2). Elsevier Inc.: 24–28. doi:10.1016/j.cell.2012.01.001.

Keating, S. T., and A. El-Osta. 2015. "Epigenetics and Metabolism." *Circulation Research* 116 (4): 715–36. doi:10.1161/CIRCRESAHA.116.303936.

Kim, Jong-yeon, Robert C Hickner, Ronald L Cortright, G Lynis Dohm, Joseph A Houmard, Robert C Hickner, Ronald L Cort-, G Lynis Dohm, and Joseph A Houmard Lipid. 2000. "Lipid Oxidation Is Reduced in Obese Human Skeletal Muscle." *American Journal* 279: 1039–44.

Kirkland, James B. 2009. "Niacin Status Impacts Chromatin Structure." *The Journal of Nutrition* 139 (12): 2397–2401. doi:10.3945/jn.109.111757.2397.

Kitano, Hiroaki. 2007. "Towards a Theory of Biological Robustness." *Molecular Systems Biology* 3 (137). doi:10.1038/msb4100179.

Knutson, Sarah K, Brenda J Chyla, Joseph M Amann, Srividya Bhaskara, Stacey S Huppert, and

Scott W Hiebert. 2008. “Liver-Specific Deletion of Histone Deacetylase 3 Disrupts Metabolic Transcriptional Networks.” *The EMBO Journal* 27 (7): 1017–28. doi:10.1038/emboj.2008.51.

Kyle, Stephanie M, Pradip K Saha, Hannah M Brown, Lawrence C Chan, and Monica J Justice. 2016. “MeCP2 Co-Ordinates Liver Lipid Metabolism with the NCoR1/HDAC3 Corepressor Complex.” *Human Molecular Genetics* 0 (0): 1–13. doi:10.1093/hmg/ddw156.

Lagos-Quintana, Mariana, Reinhard Rauhut, Abdullah Yalcin, Jutta Meyer, Winfried Lendeckel, and Thomas Tuschl. 2002. “Identification of Tissue-Specific microRNAs from Mouse.” *Current Biology : CB* 12 (9). England: 735–39.

Leung, Amy, Brian W Parks, Juan Du, Candi Trac, Ryan Setten, Yin Chen, Kevin Brown, and Aldons J Lusis. 2014. “Open Chromatin Profiling in Mice Livers Reveals Unique Chromatin Variations Induced by High Fat Diet *.” *The Journal of Biological Chemistry* 289 (34): 23557–67. doi:10.1074/jbc.M114.581439.

Li, Ping, Xiangbo Ruan, Ling Yang, Kurtis Kiesewetter, Yi Zhao, Haitao Luo, Yong Chen, and Marjan Gucek. 2015. “A Liver-Enriched Long Non-Coding RNA, lncLSTR, Regulates Systemic Lipid Metabolism in Mice.” *Cell Metabolism* 21 (3). Elsevier Inc.: 455–67. doi:10.1016/j.cmet.2015.02.004.

Linn, C, and A Srere. 1979. “Identification of ATP Citrate Lyase as a Phosphoprotein.” *The Journal of Biological Chemistry* 254 (5): 1691–98.

Lu, Chao, and Craig B. Thompson. 2012. “Metabolic Regulation of Epigenetics.” *Cell Metabolism* 16 (1). Elsevier Inc.: 9–17. doi:10.1016/j.cmet.2012.06.001.

Lynn, Francis C. 2009. “Meta-Regulation: microRNA Regulation of Glucose and Lipid Metabolism.” *Celll* 20 (9): 3–10. doi:10.1016/j.tem.2009.05.007.

Maples, Jill M, Jeffrey J Brault, Carol A Witczak, Sanghee Park, Monica J Hubal, Todd M Weber, Joseph A Houmard, and Brian M Shewchuk. 2015. “Differential Epigenetic and

Transcriptional Response of the Skeletal Muscle Carnitine Palmitoyltransferase 1B (CPT1B) Gene to Lipid Exposure with Obesity." *American Journal of Physiology. Endocrinology and Metabolism* 309 (4). United States: E345-56. doi:10.1152/ajpendo.00505.2014.

Martinez-Pastor, Barbara, Claudia Cosentino, and Raul Mostoslavsky. 2013. "A Tale of Metabolites: The Crosstalk between Chromatin and Energy Metabolism." *Cancer Discovery* 3 (5): 497–501. doi:10.1158/2159-8290.CD-13-0059.

Martìnez, Débora, Thais Pentinat, Silvia Ribò, Christian Daviaud, Vincent W. Bloks, Judith Cebrià, Nuria Villalmanzo, et al. 2014. "In Utero Undernutrition in Male Mice Programs Liver Lipid Metabolism in the Second-Generation Offspring Involving Altered Lxra DNA Methylation." *Cell Metabolism* 19 (6): 941–51. doi:10.1016/j.cmet.2014.03.026.

Mattocks, Dwight A L, Samantha J Mentch, Jelena Shneyder, Gene P Ables, Dongxiao Sun, John P Jr Richie, Jason W Locasale, and Sailendra N Nichenametla. 2016. "Short Term Methionine Restriction Increases Hepatic Global DNA Methylation in Adult but Not Young Male C57BL/6J Mice." *Experimental Gerontology* 88 (December). England: 1–8. doi:10.1016/j.exger.2016.12.003.

Mentch, Samantha J., Mahya Mehrmohamadi, Lei Huang, Xiaojing Liu, Diwakar Gupta, Dwight Mattocks, Paola G??mez Padilla, et al. 2015. "Histone Methylation Dynamics and Gene Regulation Occur through the Sensing of One-Carbon Metabolism." *Cell Metabolism* 22 (5): 861–73. doi:10.1016/j.cmet.2015.08.024.

Montgomery, Rusty L, Matthew J Potthoff, Michael Haberland, Xiaoxia Qi, Satoshi Matsuzaki, Kenneth M Humphries, James A Richardson, Rhonda Bassel-Duby, and Eric N Olson. 2008. "Maintenance of Cardiac Energy Metabolism by Histone Deacetylase 3 in Mice." *The Journal of Clinical Investigation*. doi:10.1172/JCI35847.

Moran, Victoria A, Ranjan J Perera, and Ahmad M Khalil. 2012. "Emerging Functional and

Mechanistic Paradigms of Mammalian Long Non-Coding RNAs." *Nucleic Acids Research* 40 (14): 6391–6400. doi:10.1093/nar/gks296.

Multhaup, Michael L., Marcus M. Seldin, Andrew E. Jaffe, Xia Lei, Henriette Kirchner, Prosenjit Mondal, Yuanyuan Li, et al. 2015. "Mouse-Human Experimental Epigenetic Analysis Unmasks Dietary Targets and Genetic Liability for Diabetic Phenotypes." *Cell Metabolism* 21 (1): 138–49. doi:10.1016/j.cmet.2014.12.014.

Nagaoka, Katsuya, Shinjiro Hino, Akihisa Sakamoto, Kotaro Anan, Ryuta Takase, Takashi Umehara, and Shigeyuki Yokoyama. 2015. "Lysine-Specific Demethylase 2 Suppresses Lipid Influx and Metabolism in Hepatic Cells." *Molecular and Cellular Biology* 35 (7): 1068–80. doi:10.1128/MCB.01404-14.

Nakanishi, Noriko, Yoshimi Nakagawa, Naoko Tokushige, Naohito Aoki, and Takashi Matsuzaka. 2009. "Biochemical and Biophysical Research Communications The up-Regulation of microRNA-335 Is Associated with Lipid Metabolism in Liver and White Adipose Tissue of Genetically Obese Mice." *Biochemical and Biophysical Research Communications* 385 (4). Elsevier Inc.: 492–96. doi:10.1016/j.bbrc.2009.05.058.

Nian, Hui, Barbara Delage, Emily Ho, and Roderick H. Dashwood. 2010. "Modulation of Histone Deacetylase Activity by Dietary Isothiocyanates and Allyl Sulfides: Studies with Sulforaphane and Garlic Organosulfur Compounds." *Environmental and Molecular Mutagenesis* 50 (3): 213–21. doi:10.1002/em.20454.Modulation.

Pogribny, Igor P, Sharon A Ross, Carolyn Wise, Marta Pogribna, Elisabeth A Jones, Volodymyr P Tryndyak, S Jill James, Yvonne P Dragan, and Lionel A Poirier. 2006. "Irreversible Global DNA Hypomethylation as a Key Step in Hepatocarcinogenesis Induced by Dietary Methyl Deficiency." *Mutation Research* 593: 80–87. doi:10.1016/j.mrfmmm.2005.06.028.

Potthoff, Matthew J, Hai Wu, Michael A Arnold, John M Shelton, Johannes Backs, John Mcanally,

James A Richardson, Rhonda Bassel-duby, and Eric N Olson. 2007. “Histone Deacetylase Degradation and MEF2 Activation Promote the Formation of Slow-Twitch Myofibers.” *The Journal of Clinical Investigation* 117 (9): 2459–2467. doi:10.1172/JCI31960DS1.

Rayner, Katey J, Frederick J Sheedy, Christine C Esau, Farah N Hussain, Ryan E Temel, Saj Parathath, Janine M Van Gils, et al. 2011. “Antagonism of miR-33 in Mice Promotes Reverse Cholesterol Transport and Regression of Atherosclerosis.” *The Journal of Clinical Investigation* 121 (7): 2921–31. doi:10.1172/JCI57275.eration.

Rayner, Katey J, Yajaira Suárez, Alberto Dávalos, Saj Parathath, Michael L Fitzgerald, Norimasa Tamehiro, Edward A Fisher, Kathryn J Moore, and Carlos Fernández-hernando. 2010. “MiR-33 Contributes to the Regulation of Cholesterol Homeostasis.” *Science* 328 (June): 1570–73. doi:10.1126/science.1189862.

Roberts, R, L Hodson, A L Dennis, and M J Neville. 2009. “Markers of de Novo Lipogenesis in Adipose Tissue: Associations with Small Adipocytes and Insulin Sensitivity in Humans.” *Diabetologia* 52: 882–90. doi:10.1007/s00125-009-1300-4.

Rönn, Tina, Petr Volkov, Linn Gillberg, Milana Kokosar, Anna Louisa Jacobsen, Sine W Jørgensen, Charlotte Brøns, et al. 2015. “Impact of Age , BMI and HbA1c Levels on the Genome- Wide DNA Methylation and mRNA Expression Patterns in Human Adipose Tissue and Identi Fi Cation of Epigenetic Biomarkers in Blood.” *Human Molecular Genetics* 24 (13): 3792–3813. doi:10.1093/hmg/ddv124.

Sadakierska-chudy, Anna, and Filip Małgorzata. 2015. “A Comprehensive View of the Epigenetic Landscape . Part II : Histone Post-Translational Modification , Nucleosome Level , and Chromatin Regulation by ncRNAs.” *Neurotoxicity Research* 27: 172–97. doi:10.1007/s12640-014-9508-6.

Soronen, Jarkko, Hannele Yki-Jarvinen, You Zhou, Sanja Sadevirta, Antti-Pekka Sarin, Marja

Leivonen, Ksenia Sevastianova, et al. 2016. “Novel Hepatic microRNAs Upregulated in Human Nonalcoholic Fatty Liver Disease.” *Physiological Reports* 4: 1–14. doi:10.14814/phy2.12661.

Soupene, Eric, and Frans A. Kuypers. 2008. “Mammalian Long-Chain Acyl-CoA Synthetases.” *Experimental Biology and Medicine* 233 (5): 507–21. doi:10.3181/0710-MR-287.Mammalian.

Staiger, H., N. Stefan, F. Machicao, A. Fritsche, and H.-U. Haring. 2006. “PPARGC1A mRNA Levels of in Vitro Differentiated Human Skeletal Muscle Cells Are Negatively Associated with the Plasma Oleate Concentrations of the Donors.” *Diabetologia* 49: 212–14. doi:10.1007/s00125-005-0061-y.

Sun, Zheng, Nikhil Singh, Shannon E Mullican, Logan J Everett, Li Li, Lijun Yuan, Xi Liu, Jonathan A Epstein, and Mitchell A Lazar. 2011. “Diet-Induced Lethality Due to Deletion of the Hdac3 Gene in Heart and Skeletal Muscle.” *The Journal of Biological Chemistry* 286 (38): 33301–9. doi:10.1074/jbc.M111.277707.

Tateishi, Keisuke, Yuki Okada, Eric M Kallin, and Yi Zhang. 2009. “Role of Jhdm2a in Regulating Metabolic Gene Expression and Obesity Resistance.” *Nature* 458 (7239). Nature Publishing Group: 757–61. doi:10.1038/nature07777.

Teperino, Raffaele, Kristina Schoonjans, and Johan Auwerx. 2010. “Histone Methyl Transferases and Demethylases; Can They Link Metabolism and Transcription?” *Cell Metabolism* 12 (4). Elsevier Inc.: 321–27. doi:10.1016/j.cmet.2010.09.004.

Turner, Bryan M. 2009. “Epigenetic Responses to Environmental Change and Their Evolutionary Implications.” *Philosophical Transactions of the Royal Society B: Biological Sciences* 364 (1534): 3403–18. doi:10.1098/rstb.2009.0125.

Tyagi, Sandeep, Paras Gupta, Arminder Singh Saini, Chaitnya Kaushal, and Saurabh Sharma. 2011. “The Peroxisome Proliferator-Activated Receptor: A Family of Nuclear Receptors Role in

Various Diseases." *Journal of Advanced Pharmaceutical Technology & Research* 2 (4): 236–40. doi:10.4103/2231-4040.90879.

Varvarigou, Anastasia A. 2010. "Intrauterine Growth Restriction as a Potential Risk Factor for Disease Onset in Adulthood." *Journal of Pediatric Endocrinology & Metabolism : JPEM* 23 (3). Germany: 215–24.

Vickers, Mark H. 2014. "Early Life Nutrition, Epigenetics and Programming of Later Life Disease." *Nutrients* 6: 2165–78. doi:10.3390/nu6062165.

Wahl, Simone, Alexander Drong, Benjamin Lehne, Marie Loh, William R Scott, Sonja Kunze, Pei-Chien Tsai, et al. 2017. "Epigenome-Wide Association Study of Body Mass Index, and the Adverse Outcomes of Adiposity." *Nature* 541: 81–85. doi:10.1038/nature20784.

Wang, Jinjin, Jianfeng Zhang, Jie Shen, Dongsheng Hu, Guoli Yan, Xiaohui Liu, Xueqin Xu, Lanying Pei, Yanfang Li, and Chunyang Sun. 2014. "Association of KCNQ1 and KLF14 Polymorphisms and Risk of Type 2 Diabetes Mellitus : A Global Meta-Analysis." *Human Immunology* 75 (4). American Society for Histocompatibility and Immunogenetics: 342–47. doi:10.1016/j.humimm.2014.01.008.

Weber, Michael, Ines Hellmann, Michael B Stadler, Liliana Ramos, Svante Paabo, Michael Rebhan, and Dirk Schubeler. 2007. "Distribution , Silencing Potential and Evolutionary Impact of Promoter DNA Methylation in the Human Genome" 39 (4): 457–66. doi:10.1038/ng1990.

Wellen, Kathryn E, Georgia Hatzivassiliou, Uma M Sachdeva, Thi V Bui, Justin R Cross, and Craig B Thompson. 2009. "ATP-Citrate Lyase Links Cellular Metabolism to Histone Acetylation." *Science* 324 (5930): 1076–80. doi:10.1126/science.1164097.

Woo, Melissa, and Mary-elizabeth Patti. 2008. "Diabetes Risk Begins In Utero." *Cell Metabolism* 8 (July): 5–7. doi:10.1016/j.cmet.2008.06.007.

Yang, Ling, Ping Li, Wenjing Yang, Xiangbo Ruan, Kurtis Kiesewetter, Ling Yang, Ping Li, et al.

2016. “Integrative Transcriptome Analyses of Metabolic Responses in Mice Define Pivotal LncRNA Metabolic Resource Integrative Transcriptome Analyses of Metabolic Responses in Mice Define Pivotal LncRNA Metabolic Regulators.” *Cell Metabolism* 24 (4). Elsevier Inc.: 627–39. doi:10.1016/j.cmet.2016.08.019.

Ye, Xin, Meiting Li, Tianyun Hou, Tian Gao, Wei-guo Zhu, and Yang Yang. 2016. “Sirtuins in Glucose and Lipid Metabolism.” *Oncotarget*. doi:10.18632/oncotarget.12157.

Yu, Jiujiu, Yun Li, Takahiro Ishizuka, Matthew G Guenther, and Mitchell A Lazar. 2003. “A SANT Motif in the SMRT Corepressor Interprets the Histone Code and Promotes Histone Deacetylation.” *The EMBO Journal* 22 (13). England: 3403–10. doi:10.1093/emboj/cdg326.

Zhou, Jian, and Olga G Troyanskaya. 2016. “Probabilistic Modelling of Chromatin Code Landscape Reveals Functional Diversity of Enhancer-like Chromatin States.” *Nature Communications* 7. Nature Publishing Group: 1–9. doi:10.1038/ncomms10528.

Zhou, Vicky W., Alon Goren, and Bradley E. Bernstein. 2011. “Charting Histone Modifications and the Functional Organization of Mammalian Genomes.” *Nature Reviews Genetics* 12 (1). Nature Publishing Group: 7–18. doi:10.1038/nrg2905.

Zivkovic, Angela M, J Bruce German, Farah Esfandiari, and Charles H Halsted. 2009. “Quantitative Lipid Metabolomic Changes in Alcoholic Micropigs with Fatty Liver Disease.” *Alcoholism, Clinical and Experimental Research* 33 (4). England: 751–58. doi:10.1111/j.1530-0277.2008.00892.x.

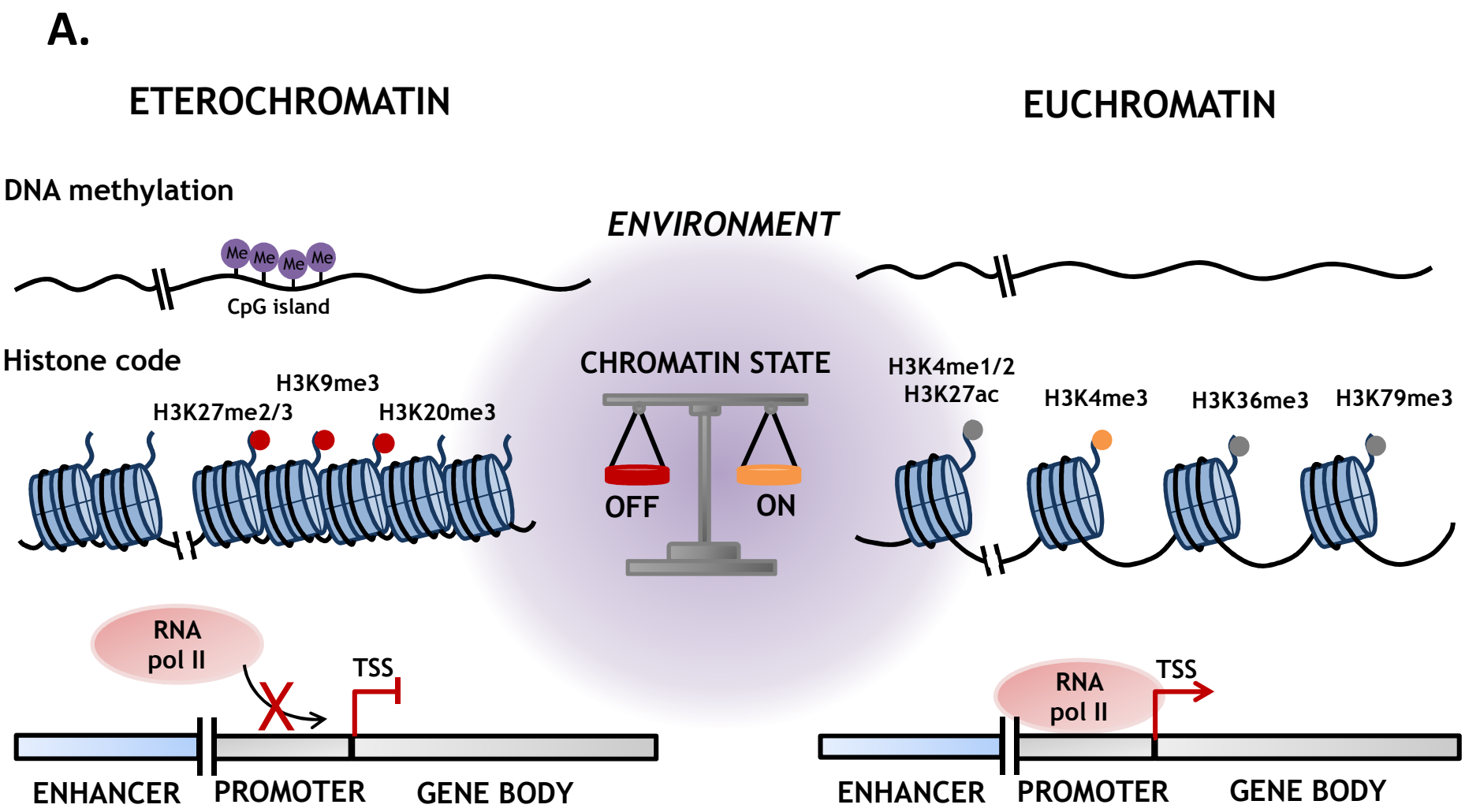
Vitae

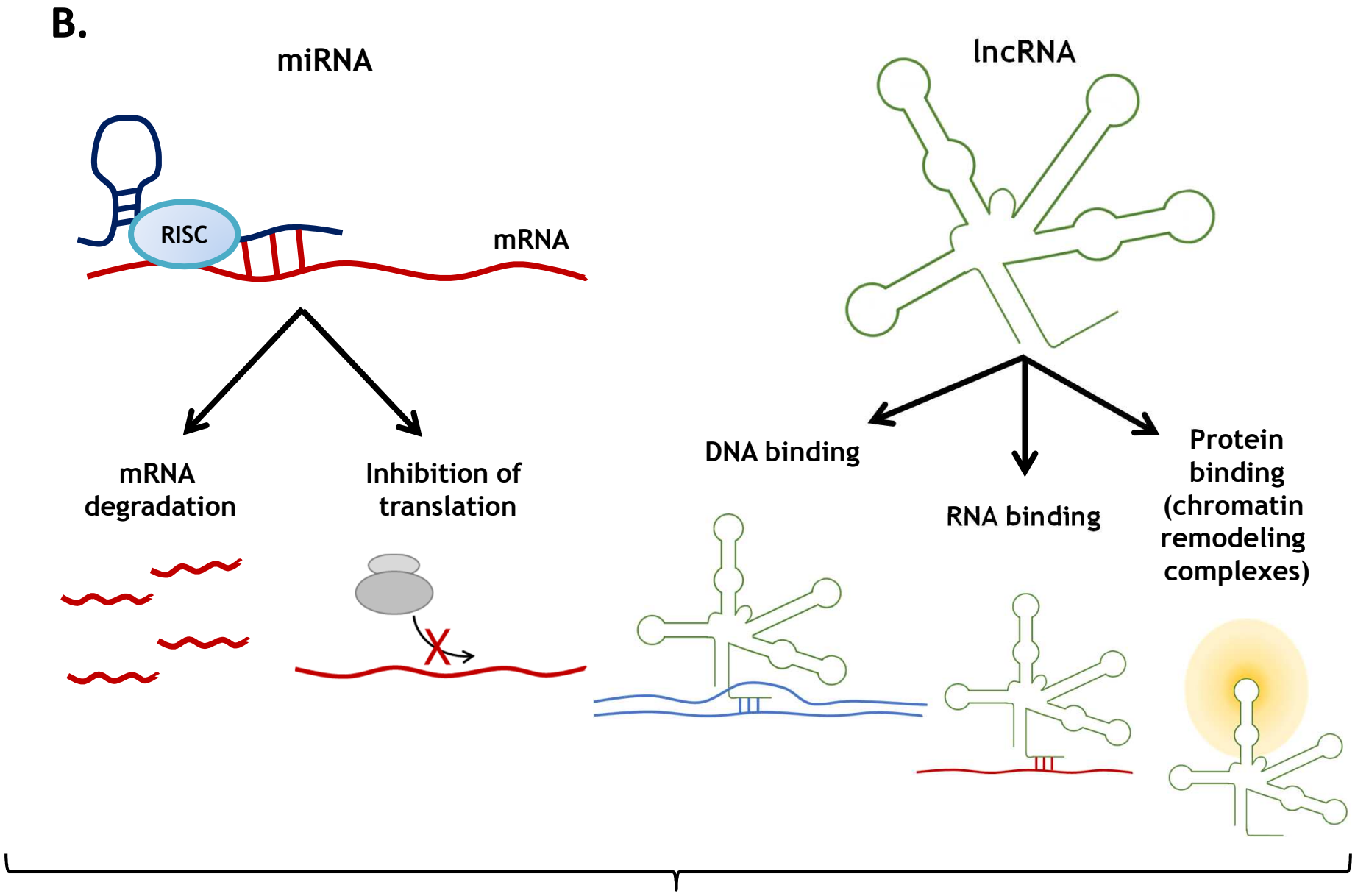
Raffaella Longo is a PhD student in Biochemistry at the Università degli Studi di Milano. She is currently studying the role of epigenome modifiers in response to cold exposure and different diets in adipose tissue.

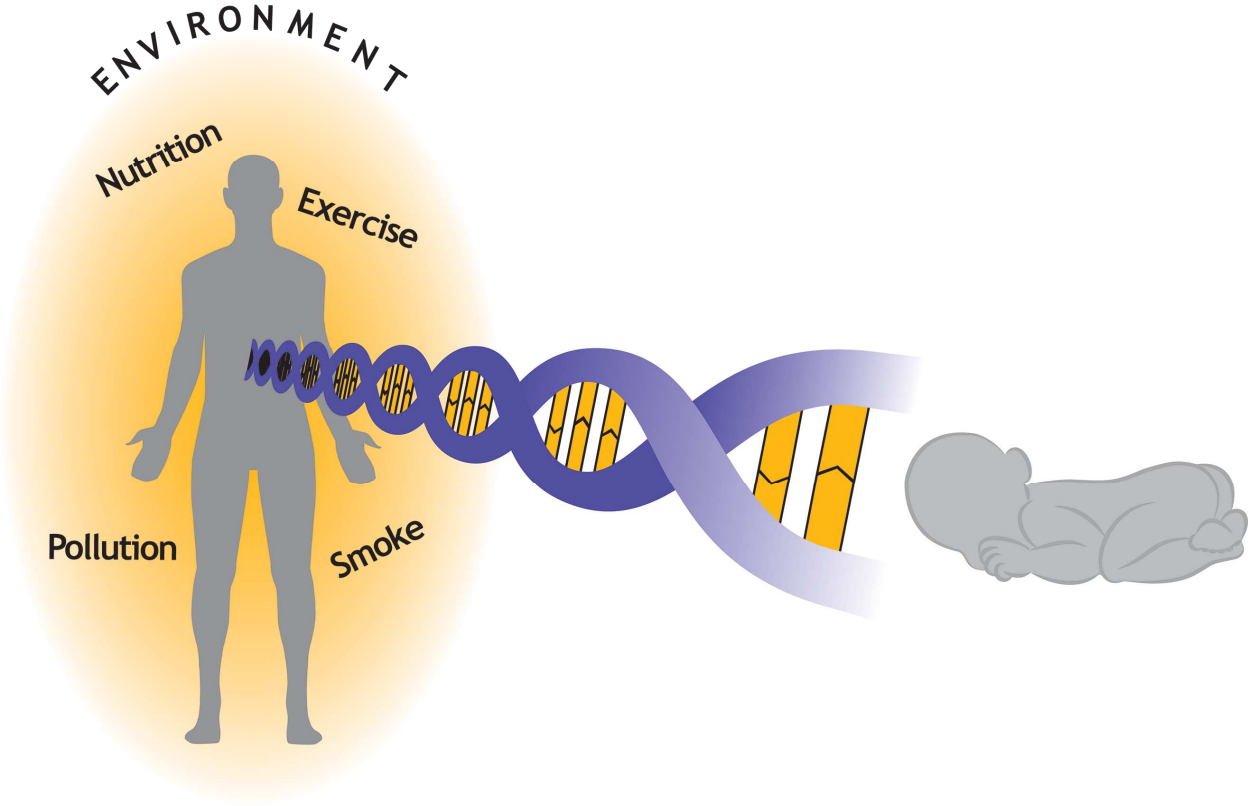
Alessandra Ferrari is a postdoctoral fellow on epigenetic regulation of adipose tissue physiology. Her main focus is on how nutrition and lifestyle affect epigenetic regulation of adipocyte differentiation, white adipose tissue metabolism and browning in the context of obesity and diabetes.

Monica Zocchi is a young fellow at the Università degli Studi di Milano. Her focus is on epigenome signatures in the liver of humanized mouse models that correlates with the phenotype of humanized mice carrying different mutations predisposing to or protecting from metabolic dysregulation predisposing to type 2 diabetes and obesity.

Maurizio Crestani is Professor of Biochemistry at the Università degli Studi di Milano. He teaches Biochemistry in the BSc program of Biotechnology and Functional Metabolic and Epigenetic Biochemistry in the MSc program of Safety Assessment of Xenobiotics and Biotechnological Products. His main interests are epigenetic regulation of energy metabolism in obesity and diabetes and novel pharmacological approaches in GLUT1 deficiency syndrome. He obtained the PhD degree at the Università degli Studi di Milano and he was a postdoctoral fellow and research instructor at the Northeastern Ohio Universities College of Medicine, Rootstown OH, studying the transcriptional regulation of bile acid synthesis.


A.
ETEROCHROMATIN
EUCHROMATIN
DNA methylation
ENVIRONMENT
Me
Me
Me
Me
CpG island
Histone code
CHROMATIN STATE
H3K9me3
H3K27me2/3
H3K20me3
H3K4me1/2
H3K27ac
H3K4me3
H3K36me3
H3K79me3
OFF
ON
RNA
pol II
TSS
RNA
pol II
TSS
ENHANCER
PROMOTER
GENE BODY
ENHANCER
PROMOTER
GENE BODY


B.
miRNA
RISC
mRNA
mRNA degradation
Inhibition of translation
lncRNA
DNA binding
RNA binding
Protein binding (chromatin remodeling complexes)
GENE EXPRESSION REGULATION



Mouse

Human

DNA methylation

Differentially **DNA methylation regions** conserved across species
625 genome-wide DMRs in murine adipocytes that correlated with diet-induced obesity.
Among these, 249 presented conserved methylation changes in human obesity
170 had the same direction of methylation changes in both species.
30 of these DMRs overlapped with SNP associated with human T2D genetic risk.

In WAT from **high fat diet** fed mice
- Upstream to TSS of *Pparg* gene (master regulator of adipose function)
- In *Pck1* promoter (glyceroneogenesis)

In utero undernutrition of male mice (F1) reduces lipogenic genes (Acly, Fasn, Scd1, Elovl6 and Srebf1) in the liver of their offspring (F2). as result of reduced expression of master regulator of liver lipogenesis, Lxr-a, showing
- reduced DNA methylation level at CpG 5' UTR of promoter region
- enrichment of repressive histone marks H3K9me2 and H3K27me3

Obesity-associated methylation at CpG sites in blood cells on lipid-related genes
- *CPT1A* (mitochondrial uptake of long-chain fatty acids)
- *ABCG1*(macrophage cholesterol and lipid homeostasis)
- *SREBF* (lipogenesis)

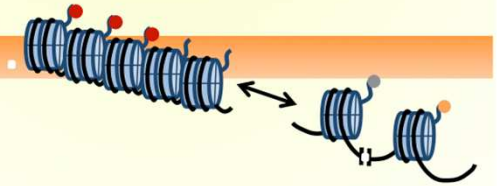
Obesity blunts the reduction in cytosine methylation in *CPT1B* promoter (FA β-oxidation) in response to lipid overload in muscle cells

Correlations between **age** and methylation in CpG sites in *ELOVL2* gene (lipid metabolism) in blood and adipose cells

NASH determined decreased DNA methylation and consequent higher expression of *ACLY* gene (lipogenesis)

Exposure to **famine during early gestation** correlates with changes in DNA methylation in genomic portion of *INSR* (insulin receptor) and *CPT1A*

Histone modification

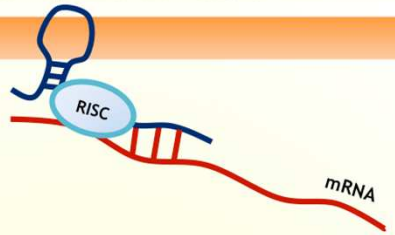


Treatment of in primary hepatocytes with fatty acids (resembling **hepatosteatosis**) altered H3K4me3 and H3K9me3 profile
- *Ppara* promoter (lipid catabolism)

Genetic ablation of H3K9-specific demethylase Jmjd1a impaired metabolism and induced obesity

Lysine-specific demethylase 2 (LSD2) regulates lipid metabolism in human hepatocytes: demethylating H3K4, LSD2 is able to repress genes involved in lipid influx and metabolism

Micro RNAs



MiR-33 suppresses the expression of
- cholesterol ATP-binding cassette A1 (Abca1)

and other genes involved in cholesterol transport
- Srebf1c (de novo fatty acid synthesis)

MiR-122 regulates cholesterol and fatty acid metabolism in the liver
- MiR-122 inhibition decreased expression of genes involved in fatty acid synthesis (*Fasn*, *Acc1*, *Acc2*, *Scd1*, and *Acly*).

MiR-122 is involved in lipogenic regulation and is reduced in NASH, leading to increased protein levels of its targets (FASN, HMGCR) and their activators (SREBP-1c, SREBP-2)