Epigenetic modifications in adipose tissue – relation to obesity and diabetes

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Abstract
The growing number of people suffering from obesity and type 2 diabetes mellitus (T2DM) is a global health problem that results in increased mortality from their complications, mainly cardiovascular diseases. Although the relationship between obesity and T2DM is well established, the common molecular pathomechanisms are still under investigation. Recently, it has been suggested that epigenetic modifications may be involved in both obesity and T2DM development. Epigenetics plays a pivotal role in the regulation of gene expression by the reversible modifications of chromatin structure without any changes in DNA sequence. Epigenetic modifications include DNA methylation, posttranslational histone modifications and miRNA interference. Therefore, the aim of this article is to discuss the current knowledge on epigenetic modifications in adipose tissue and their association with obesity and T2DM.

Key words: epigenetic modifications, adipose tissue, obesity, type 2 diabetes mellitus.

Introduction
According to the World Health Organization, obesity and type 2 diabetes mellitus (T2DM), which frequently occur together (diabesity), have become a global 21st century epidemic. Both obesity and T2DM lead to serious life-threatening complications, especially cardiovascular diseases. Epidemiological data show that between 60% and 90% of T2DM patients are overweight or obese [1]. Moreover, Ford et al. suggest that one kilogram of weight gain increases the risk of T2DM development by 4.5% to 9% [2]. However, it is ambiguous whether obesity is a risk factor or a cause of T2DM development. It was found that the development of obesity and/or T2DM may be associated with changes in gene expression evoked by genetic and environmental factors [3]. Moreover, it was demonstrated that gene expression is also under the control of epigenetic mechanisms responsible for switching between active and inactive states of chromatin [4].

Notable evidence demonstrating the involvement of epigenetic mechanisms in the development of metabolic disorders comes from the “Dutch famine birth cohort” study. In this study, children of Western Netherlands’ women, affected by the Nazi embargo, born between November 1943 and February 1947, have been followed up since 1996. Children con-
ceived by malnourished women (caloric intake of 400–800 per day) were smaller and more prone to develop several chronic diseases, including cardiovascular disease, obesity, diabetes, elevated blood pressure, and impaired glucose tolerance in later life [5]. Noteworthy, other observational studies showed that children conceived by mothers with gestational diabetes were also more susceptible to obesity, T2DM or metabolic syndrome [6]. Taken together these observations suggest that the fetal developmental environment may evoke significant epigenetic modifications that predispose the offspring to metabolic diseases (Figure 1).

Twin studies are considered as a crucial approach to unravel the interplay between heritable and environmental factors in the etiology of various diseases, including metabolic disorders [7]. These studies provide an opportunity to separate the influence of common environmental from genetic factors on disease phenotype by comparing monozygotic twins to dizygotic twins. Monozygotic twin studies are considered as unbiased due to the fact that they are genetically identical (age, sex) and share congruent environmental influences (cohort effects, maternal influences and common environment), whereas dizygotic twins share on average 50% of genetic variants [8]. The analysis of cardiometabolic risk factors using a structural equation model performed on 63 monozygotic and 38 dizygotic adult twins revealed that some risk factors were strongly associated with heritability (weight, waist circumference), whereas others (fasting insulin, fasting blood glucose and total cholesterol) were significantly influenced by the environment [9]. Therefore, it seems that twin studies give an excellent opportunity to assess the influence of environment and heritability on epigenetic pattern and related disease phenotype.

The main goal of this manuscript is to present contemporary findings related to epigenetic modifications in adipose tissue and their association with obesity and type 2 diabetes.

**Epigenetics – definition and background**

The term “epigenetics” was introduced in the 1940s by Waddington, as an attempt to define the role of genetics in developmental processes [10]. Nowadays, epigenetics is a discipline exploring gene expression involving functional changes in chromatin structure and non-coding RNAs without direct change in nucleotide sequences of DNA. Epigenetic regulation includes modifications of chromatin proteins, methylation of DNA and miRNA interference. The local packing of chromatin, loose (euchromatin) or tight (heterochromatin), determines the access of proteins responsible for gene expression. Euchromatin, a transcriptionally active form of chromatin, consists of nucleosomes (DNA double-helix DNA wrapped around the octamer of core histone proteins: dimers of H2A, H2B, H3 and H4 histones) connected via internucleosomal DNA, which is known as ‘beads on a string’. Heterochromatin, an inactive form of chromatin, arises as a result of H1 histone interaction with nucleosomes. Epigenetic modifications of DNA and core histone proteins may weaken or strengthen the interaction between nucleosomes and H1 histones, which results in loosening or tightening of chromatin structure, altering the access of proteins to genes [4]. Epigenetic changes were found to be involved in developmental and differentiation processes, i.e. repetitive elements silencing, inactivation of chromosome X during sex determination, and genomic imprinting during gametogenesis. A growing body of evidence suggests that changes in the epigenome induced by the environment may alter gene expression and lead to the development of various diseases, including cancers, obesity and diabetes [11].

DNA methylation is a reversible process performed by DNA methyltransferases (DNMTs) and DNA demethylases (DNAdeMeths). Methylation of DNA involves the addition of a methyl group to cytosine in specific regions, called CpG islands. Areas of lower transcriptional activity were detected as highly methylated, while hypomethylation of cytosine was associated with increased gene expression [12].

Posttranslational modifications of core histones include acetylation, methylation, phosphorylation, ubiquitination, and sumoylation [13]. Currently, much attention is focused on core histones’ methylation and acetylation of lysine and argi-
niece. Key enzymes responsible for modifications of N-terminal tails of H2A, H2B, H3 and H4 are histone acetyltransferases (HATs), histone deacetyltransferases (HDACs), histone methyltransferases (HMTs) and histone demethylases (HDMs) [11]. The effect of acetylation and methylation on the regulation of gene expression was found to be dependent on the site of modification. It was reported that acetylation of H3 histone at lysine-9/14 sites (H3KAc) and methylation of H3 histone at lysine-4 (H3K4me) were associated with activation of gene expression. Contrariwise, methylation of H3 histone at lysine-9 (H3K9me) or methylation of H3 histone at lysine-27 (H3K27me) was demonstrated to inhibit gene expression. HMTs were shown to mediate the addition of two or three methyl groups to a specific lysine residue [14].

Interestingly, enzymes involved in histone epigenetic modifications were also demonstrated to modify non-histone proteins, such as nuclear factor-κB (NF-κB) and p53 [15]. Both NF-κB and p53 are transcription factors, engaged in the control of cell survival and the inflammatory process.

In addition to chromatin structure modification, another mechanism of epigenetic regulation is gene silencing through single-stranded non-coding RNAs [16]. miRNAs are small molecules, approximately 19 to 33 nucleotides in length, derived from endogenous transcripts generating a hairpin structure, that interact with specific regions of mRNA. Pairing between miRNA and mRNA results in translation inhibition, as a result of degradation of mRNA and/or destabilization of mRNA structure [17, 18]. Recent evidence identified involvement of various miRNAs in the pathogenesis of several diseases, including obesity and diabetes.

Adipose tissue as a site of initiation of metabolic disorders

Adipose tissue contains different types of cells, including mainly adipocytes and a stromal vascular fraction composed of fibroblasts, preadipocytes, vascular endothelial cells and immune cells (macrophages, lymphocytes) [19]. Apart from energy storage, cushioning and insulating the body, adipose tissue was established as an important endocrine organ, secreting various hormones, adipocytokines (adipokines) and free fatty acids (FFAs) [20]. Adipocytes and adipose tissue macrophages express various receptors including peroxisome proliferator-activated receptors γ (PPARγ), fundamentally important for adipose tissue function. The PPARγ are crucial for differentiation and apoptosis of adipocytes, as well as the regulation of lipogenesis-related genes. Additionally, activation of PPARγ is a part of the regulation of adipocytokine expression, including leptin and tumor necrosis factor α (TNF-α) and interleukin-6 (IL6) [21].

Adipose tissue is situated in various body departments: under the skin (subcutaneous fat), around internal organs (visceral fat: mesenteric, epididymal white adipose tissue, and perirenal depots), bone marrow (yellow fat marrow) and in breasts. Excess of visceral adipose tissue (VAT), also called central obesity, is considered an essential factor for the development of glucose intolerance and T2DM associated with insulin resistance (tissues’ impaired ability to respond properly to insulin action) [22, 23]. Adipose tissue constantly deals with FFA turnover. Whether FFAs will enter the adipocyte, being reassembled in the form of triglycerides, or leave the cell (lipolysis) is controlled by the relation between insulin and leptin level [24]. Leptin is an adipocytokine that stimulates the inflammatory response and prevents lipid accumulation in adipose tissue, while high insulin levels exhibit an antilipolytic effect, promoting the storage of triglycerides in adipocytes [25]. In obese individuals, hypertrophic visceral adipocyte cells have a lower density of insulin receptors and become resistant to insulin, exhibiting a hyperlipolytic state. As a result, FFAs are fluxed into the liver, which in turn impairs liver metabolism, leading to glucose overproduction [24]. Chronically elevated levels of glucose and lipids exert toxic effect (gluco- and lipotoxicity) on various organs, resulting in serious metabolic disorders [26]. Additionally, hypertrophic adipocytes facilitate the migration of macrophages to the stromal fraction, initiating and promoting the inflammatory process [27]. Moreover, the enhancement of obesity-related chronic low grade inflammation is promoted via FFA oxidation in the liver, which results in reactive oxygen species (ROS) generation [28]. Reactive oxygen species production by macrophages and monocytes is also stimulated by pro-inflammatory cytokines. It was recognized that the secretory profile of adipose tissue in overweight/obese people is altered [29]. The expression of pro-inflammatory cytokines (TNF-α, IL-6) increases, whereas expression of cytokines improving insulin sensitivity (e.g. adiponectin) is reduced. It is well known that both oxidative stress and chronic low-grade inflammation having an impact on insulin secretion and insulin sensitivity may exaggerate the development of T2DM and atherosclerosis-associated diseases.

Epigenetic modifications in adipose tissue

DNA methylation pattern

Studies indicate the involvement of DNA methylation in differentiation of adipocytes and its effect on expression of genes related to obesity and diabetes.
One major study exploring the role of DNA methylation in adipogenesis was conducted by Chapman et al. They found that treatment of mouse embryonic fibroblast (10T1/2) with an inhibitor of mammalian DNA methylation, 5-aza-cytidine, gave rise to new cell types, including preadipocytes (TA1, TA2) featuring the accumulation of lipid droplets. Additionally, insulin- and dexamethasone-induced differentiated TA1 cells showed changes typical for mature adipocytes. A significant increase in glycerol phosphate dehydrogenase activity and the level of proteins involved in fatty acid and triglyceride synthesis, as well as changes in total mRNA expression, was observed [30]. Wabitsch et al. reported that using 5-aza-deoxycytidine, a DNA hypomethylation agent, during differentiation of a cell line derived from liposarcoma (LiSa-2), resulted in a significant increase in leptin mRNA expression [31].

Recently published results of the study performed by Malodobra-Mazur et al. on differentiated 3T3-L1 adipocytes suggested that the level of proinflammatory cytokine expression might be regulated by stearoyl-CoA desaturase 1 (SCD1) through DNA methylation [32]. SCD1, an enzyme highly expressed in differentiated adipocytes, is responsible for the biosynthesis of monounsaturated fatty acids from saturated fatty acids. It is suggested that SCD1 may be involved in obesity-related inflammation [33]. Findings indicate that over-expression of SCD1 is associated with global DNA hypomethylation, whereas silencing or inhibition of SCD1 evokes global hypermethylation. Additionally, it was found that the promoter methylation pattern in CpG islands of interleukin-10 receptor a (IL10ra), interleukin-4 receptor a (IL4ra), interleukin-6 signal transducer (IL6st), and transforming growth factor β1 (TGFβ1) genes, was changed by SCD1 overexpression and correlated with changes in expression of the IL10ra, IL4ra, TGFβ1, and IL6st genes [32].

Bouchard et al. investigated the DNA methylation pattern and changes in gene expression in subcutaneous adipose tissue of obese/overweight postmenopausal women before and after a 6-month caloric restriction diet [34]. Participants were grouped into high and low diet responders according to changes of body fat percentage after completion of the experiment. Differences in DNA methylation between high and low responders were observed at 35 loci before and at 3 loci after dietary intervention. Several loci comprised genes involved in the control of weight (potassium voltage-gated channel shaker-related subfamily member 3 [KCNA3], nuclear factor I/X [NFI/X]), insulin secretion and diabetes (insulinoma-associated 1 [INSM1], GLIS family zinc finger 3 [GLIS3]), cholecystokinin B receptor [CCKBR]). It was found that gene expression changes appeared only after dietary intervention, and included 644 genes engaged in metabolic pathways (327 genes up-regulated and 317 down-regulated). Comparing high diet responders to low diet responders, the highest overexpression was observed for the gene encoding cholesteryl ester transfer protein (CETP) and the highest downexpression was found for phosphoserine phosphatase (PSPH) mRNA. The CETP is involved in cholesteryl ester and triglyceride transfer among plasma lipoproteins, whereas PSPH is responsible for L-serine formation. Recently, CETP was also proposed to play a role in metabolism and storage of lipids in adipose tissue [35].

The relationship between 8-week low-caloric diet and methylation level in the promoter regions as well as the expression level of leptin and TNF-α determined in human subcutaneous adipose tissue was assessed by Cordero et al. [36]. Subjects were divided into responder and non-responder groups on the basis of changes in weight, lipid profile, fat mass percentage, and systolic and diastolic blood pressure after diet completion. A lower methylation pattern in the promoters of leptin and TNF-α detected before starting the diet was found in responders as compared to non-responders, but there were no differences after diet completion. The level of mRNAs for leptin and TNF-α did not differ significantly between responders and non-responders before implementation of the diet. However, after dietary intervention leptin expression level was significantly decreased in the responder group, but no changes in TNF-α expression level were observed. These observations suggested that methylation level of leptin and TNF-α promoters may be an epigenetic predictor of responsiveness to a caloric restriction diet.

Rönn et al. [37] examined the effect of 6-month exercise intervention on DNA methylation pattern at 476,753 CpG sites in adipose tissue of healthy men previously representing low physical activity. They found that the global DNA methylation pattern was altered at 17,975 CpG sites in response to exercise. The authors also observed that after exercise adipose tissue displayed differential methylation at 236 CpG sites of 197 gene regions along with a distinct alteration in mRNA expression of these genes. Moreover, the relation between DNA methylation and expression of obesity and T2DM candidate genes was explored. Among 476,753 CpG sites after exercise, 24 CpG sites with a difference in DNA methylation were located in 18 candidate genes for obesity, whereas 45 differentially methylated CpG sites were observed in 21 T2DM candidate genes. However, changes in gene expression in parallel with differ-
ential DNA methylation were found only for 2 obesity candidate genes (cytoplasmic polyadenylation element binding protein 4 [CEBP4], serologically defined colon cancer antigen 8 [SDCCAG8]) and 4 T2DM-related genes (hemato poietically expressed homeobox [HHEX], insulin-like growth factor 2 mRNA binding protein [2IGF2BP2], juxtaposed with another zinc finger gene 1 [JAZF1], and transcription factor 7-like 2 [TF7L2]). Of note, 2 genes (histone deacetylase 4 [HDAC4], nuclear receptor co-repressor 2 [NCOR2]) exhibiting increased DNA methylation accompanied by decreased mRNA expression in adipose tissue after exercise were selected to assess their effect on adipocyte metabolism. HDAC4 plays a significant role in the repression of glucose transporter 4 (GLUT4) transcription in adipocytes, whereas NCOR2 regulates the expression of genes involved in lipid metabolism and adipogenesis, as well as recruitment of various histone deacetylases, including HDAC4. As expected, siRNA-induced silencing of HDAC4 and NCOR2 resulted in increased lipogenesis in 3T3-L1 adipocytes.

Posttranslational core histone modifications

Among investigated posttranslational core histone modifications in adipose tissue, the largest body of evidence has accumulated for H3 histone.

Steger et al. found that the process of preadipocyte differentiation was associated with transient epigenetic modification of H3 histone [38], 3T3-L1 preadipocytes were induced to differentiate into mature adipocytes by the treatment with insulin, glucocorticoids and cAMP signaling inducers. H3 histone modifications (H3K4 trimethylation – H3K4me3, H3K4 dimethylation – H3K4me2, and H3K9 acetylation – H3K9ac) overlapping 74 gene regions were investigated at days 0, 1, and 10 of 3T3-L1 differentiation. Among these genes’ regions, 20 exhibited increased levels of H3K4me3, H3K4me2 and H3K9ac at the end of differentiation (day 10), as compared to undifferentiated cells (day 0). Of note, only PPARγ regions showed increased levels for all three modifications on the first day of differentiation. The induction of PPARγ expression was detected ∼24 h after the initiation of differentiation and was sustained in mature 3T3-L1 adipocytes. Steger et al. found that PPARγ transactivation was a consequence of a transient assembly of glucocorticoid receptor (GR) and CCAAT/enhancer-binding protein β (CEBPβ) with p300 and mediator subunit 1 (MED1), stimulating histone H3 acetylation at several enhancers, including enhancers for PPARγ. Importantly, this assembly occurred only at the start of adipogenesis (day 1).

Tateishi et al. demonstrated that genetic knockout of specific H3K9 demethylase, jumonji C-domain containing protein (Jhdm2a), increased mice susceptibility to obesity, as a result of a decrease in PPARα expression in skeletal muscles and brown fat [39]. Jhdm2a deficiency led to abnormal fat distribution and hyperlipidemia. It was also found that other genes involved in the PPAR pathway were down-regulated, including those participating in fatty acid metabolism (uncoupling protein 2 [UCP2], medium-chain acyl-CoA dehydrogenase [MCAD], light-chain acyl-CoA dehydrogenase [LCAD], very long chain acyl-CoA dehydrogenase [VLCAD]) and glyceroi release (aquaporin 7, [Aqp7]).

Okuno et al. investigated the role of another jumonji C-domain containing protein responsible for demethylation of H3K9me2 (plant homeodomain finger 2 [PHF2]; also called JHDM1E) in the process of adipogenesis [40]. They generated transgenic PHF2-knockout mice that exhibited a partial neonatal death, growth delay, and reduction of body weight. Moreover, transgenic mice showed lipotoxic changes, mainly in epididymal and subcutaneous white adipose tissue and slight lipoatrophy in mesenteric adipose tissue. In vitro studies on mice 3T3-L1 fibroblasts and vascular stromal cells with knockout of PHF2 induced to differentiate into mature adipocytes showed impairment of adipogenesis. It was reported that PHF2 was a coactivator of metabolism-related transcription factors through interaction with CEBPγ and demethylation of H3K9me2 in the promoters of CEBPγ-regulated adipogenic genes, including PPARγ, CEBPγ, and fatty acid binding protein 4 (FABP4) [40].

Hino et al. demonstrated that inhibition of lysine-specific demethylase-1 (LSD1) – via siRNA and selective inhibitors – increased expression of metabolism-related genes (PPARγ coactivator-1α [PGC-1α], pyruvate dehydrogenase kinase 4 [PKD4], protein kinase A regulatory subunit 2α [Rlk2], adipose triglyceride lipase [ATGL], fatty acid transporter protein 1 [FATP1]) in 3T3-L1 differentiating adipocytes [41]. Transcription repression by LSD1 is associated with removal of a methyl group from mono- and dimethylated K4 of H3. They also reported that the inhibition of LSD1 activity in differentiating 3T3-L1 cells led to a significant increase in mono-methylated K4 of H3 (H3K4me1) and enhanced mitochondrial metabolism (detected by increased mitochondrial membrane potential and increased mitochondrial oxidative phosphorylation). Results from experiments performed on mice demonstrated that consumption of a high fat diet for 6 weeks induced an obese phenotype with the increase of LSD1 expression and decreased expression of PGC-1α, PD4K, FATP1 in adipose tissue.

Enhanced H3K4me2 in promoters/enhancers, transcribed regions and increased expression of
A growing amount of evidence indicates that miRNAs play a crucial role in the regulation of cellular metabolism in adipose tissue, and in the pathogenesis of obesity and diabetes. Several miRNAs have been identified as being involved in these processes. For example, miR-483-3p has been shown to be downregulated in obese adipose tissue compared to lean tissue. This miRNA has been linked to the regulation of adipocyte differentiation and the development of insulin resistance. Another important miRNA is miR-143, which is upregulated in obese adipose tissue and has been shown to inhibit adipocyte differentiation.

In conclusion, miRNAs play a significant role in the pathogenesis of obesity and diabetes. They are involved in the regulation of cellular metabolism in adipose tissue, and in the development of insulin resistance. Further research is needed to fully understand the role of miRNAs in these processes and to develop potential therapeutic strategies to target these miRNAs.
Due to the incompletely understood pathogenesis of obesity and T2DM, it is necessary to search for genetic and environmental factors that may have an impact on the development and progression of these metabolic disorders. Findings from this research may indicate new targets for the prevention and treatment of these conditions. A growing number of both experimental and clinical studies suggest that epigenetic modifications may become such targets. This hypothesis is at least partially supported by the results of randomized clinical trials showing that early intensive glycemic control in diabetic patients might delay the development of diabetes and its complications. Early normalization of glycemia may induce epigenetic changes affecting gene expression and in consequence reduce hyperglycemia-associated oxidative stress and advanced glycation of protein and lipids. Therefore, further research to explore epigenetic mechanisms may reveal important information for the pathogenesis, treatment and prevention of diabesity.

**Conflict of interest**

The authors declare no conflict of interest.

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