

**MANIPULATION OF EPIGENOME: OPPORTUNITIES AND PITFALLS IN FIGHTING AUTOIMMUNE DISEASES****H. Higazi<sup>1</sup>, R.P.Kotian<sup>2</sup>, F. A. Khan<sup>3</sup>, A. S. Yousif<sup>4</sup>, A. Ibrahim<sup>5</sup>, S. Ali<sup>6</sup>, A.E. Altoum<sup>7</sup>, N. Patil<sup>8</sup>, A. Hazari<sup>9</sup> and S. Gurung<sup>10</sup>**<sup>1\*,2,6,7,9</sup> College of Health Sciences, Gulf Medical University, Ajman, United Arab Emirates<sup>3</sup> University of Central Punjab, Lahore, Pakistan<sup>4</sup> The Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology and Harvard University, 400 Technology Square, Cambridge, MA 02139, USA<sup>5</sup> Tongji Medical College, Huazhong University of Science and Technology Wuhan 430030, China.<sup>8,10</sup> Department of Pharmacology, United College of Medical Sciences, Bhairahawa, Nepal.<sup>1</sup>dr.hassan@gmu.ac.ae, <sup>2</sup>dr.rahul.k@gmu.ac.ae, <sup>3</sup>faheemgenetics@yahoo.com, <sup>4</sup>ashraf.siddig@drug-farm.com, <sup>5</sup>asjatibrahim@yahoo.com, <sup>6</sup>dr.sara@gmu.ac.ae, <sup>7</sup>dr.abdel@gmu.ac.ae, <sup>8</sup>navin903@gmail.com, <sup>9</sup>dr.animesh@gmu.ac.ae, <sup>10</sup>sonupaigilamagr7@gmail.com**ABSTRACT**

Many recent studies have focused on the manipulation of the epigenome to understand the mechanistic programming in health and some disease phenotypes. These studies are designed to provide suitable drug targets to cure and/or prevent the outcome of a disease condition. Autoimmune diseases including obesity and diabetes are of major health concern nowadays and are the root cause of several diseases of the heart, lungs, and liver. There are several epigenetic mechanisms underlying the manifestation of autoimmune disorders. The recent advances in today's sequencing technology and genome editing have uncovered the role of epigenetic modifications in autoimmune diseases. In this review we will cover the recent discoveries and its possible application for the control of autoimmune diseases with improving the long-term use of such technologies. The potential drawbacks will also be discussed so that future experiments may be designed to reduce or eliminate the risk factors associated with the use of recent discoveries in the field of medicine.

**Introduction**

History has witnessed several famines which cause malnutrition in the human population thereby leading to several diseases including anorexia; however, recently obesity has taken the world by surprise and has become one of the leading causes of life threatening diseases (1). This long history of starved conditions resulted in the evolution of an organ termed adipose tissue to provide necessary nutrition at times of need (1). Such an event of evolution resulted in better regulation of energy via the central nervous system through a set of defined signaling pathways as well as it has a self-system of recognizing energy demand and supply requirements (2). Over time the human lifestyle has changed a lot and the fat tissues stored in the body causes overweight and obesity (3). The function of adipose tissue in the era of under-nutrition and over-nutrition is distinct and requires special attention before it causes damage to other organs and tissues.

The obesity epidemic is currently posing a significant threat because it affects children and young adults, compromising their life expectancy by nearly one half due to an increase in obesity-related disease burden (4).

Obesity can be defined in terms of body mass index (BMI) where  $> 30$  BMI value is considered obese whereas  $>25$  is taken as overweight (5). In the evolutionary context, the genotypes that support the energy storage are more suited to survive in the age of famine and low food availability; however, recent developments in agriculture and the food industry resulted in an epidemic of obesity that is strongly associated with an increase in incidence of several diseases, including diabetes type 2, hypertension, heart ischemia and other metabolic diseases (4). Such genotypes termed as "thrifty genotypes" (6) became un-adaptive in recent times of low energy expenditures and is strongly associated with type 2 diabetes. It was Neel who correlated obesity to sickle cell anemia of which allele was beneficial to carry at certain times of adaptation and survival (7). While, Neel's hypothesis is partially acceptable; Hales and Barker later showed that malnutrition in uterus actually causes disturbance in glucose tolerance and other metabolic syndromes which is supported by epidemiological data (8). However, more recently both hypotheses are being challenged with predator theory, which

relates it to a genetic drift because of the absence of predators in the recent era. The epigenetic changes in relation to the autoimmune diseases, obesity and diabetes have been recently studied, and several target proteins have been recommended for anti-obesity and anti-diabetic targets. This review will go through the major findings and recent day biological questions in context of autoimmune, obesity and diabetes and pose it scientific community for further research and development.

### Countering Obesity and diabetes

Obesity is one of the major public health issues of the current era (9), associated with several major diseases of vital organs such as heart, lung and kidney, affecting life sustaining systems such as circulation, breathing and excretion, causing mortality and morbidity (10). Obesity is strongly associated with cancers; however, its molecular mechanism was vague until Hao *et al.* discovered that circulating adipose fatty acid binding protein (A-FABP) is the cause for promoting breast cancer by direct interaction with tumor cells and activates IL-6/STAT3/ALDH1 pathway (11). According to the WHO, 1.9 billion adults of age 18 and above are overweight, out of which 650 million are obese. The problem continues in children, and 41 million children under age 5 are reported to be overweight or obese (12). Much of the world's population is at risk of developing type 2 diabetes because of easily available high caloric foods which are now the routine diet of many. This includes children and adolescents, exposing them to the future threat of energy intake and expenditure imbalance (13). Advanced research in understanding the mechanism and pathways associated with obesity and its cure is a very important need of the moment, several labs are demonstrating obesity and its linkage to epigenetic modulation and its interaction with diets and environmental factors is becoming clearer.

Gene expression is considered at the core of the obesity problem, which is controlled by several epigenetic mechanisms including DNA methylation, histone acetylation, and phosphorylation affecting key pathways responsible for maintaining energy

homeostasis. Today basic and advanced molecular, immunological and biotechnological procedures help diagnose several of the important mechanistic insights into previously unknown phenomena (14). The following will illustrate the recent advancements in obesity research with a focus on epigenome.

Insulin resistance is the major cause of obesity that leads to imbalance in energy homeostasis and has been the target focus of research. It is well established that chronic inflammation of the adipose tissue, skeletal muscles and liver is the major cause of obesity (15), with a critical role played by miRNAs and exosomes (16). Exosomes are the micro-vesicles that have the capability to carry miRNAs, of which the adipose tissue secreting exosomes are shown to carry miR155 (17) of which PPAR- $\gamma$  is the target gene, and of which miR155 KO mice is shown to have high insulin sensitivity and glucose tolerance. It also demonstrates the ability of exosomes to carry the miRNAs of choice to target cells and tissues to therapeutically treat insulin sensitivity and glucose tolerance (16). The induction of white adipose tissue to brown or increasing the number of brown adipocytes that can dissipate energy is another approach to overcome obesity outbreak and is a major area of diabetes and obesity research (15). Recently, in mouse inguinal WAT tissues, researchers observed neural abrasions at single-fiber level and uncover how the sympathetic nervous system and CNS play a role in the architecture of adipose tissue (18). The efficient induction of beiging in humans remains a problem, where a recent study showed how intermittent fasting can induce beiging of white adipose tissue and help improve diabetes (19).

The induction of beiging, however, require activation of  $\beta$ -adrenergic receptors mostly by cold induction which is why no successful therapeutic agent can be introduced until now as  $\beta$ -adrenergic signaling pathway is involved in several functioning of tissues and raises safety concerns. This requires a safe way to induce thermogenic fat cells that can burn fat to reduce weight without causing harm to the body. To this end, Chen *et al.* (18) discovered a fat cell that burns only glucose and termed them as glycolytic beige cells (20). This

discovery opens a new avenue to combat obesity as it follows a previously uncharacterized pathway. If such cells are found in humans, it can help develop new therapeutic avenues.

**1. Epigenetics of Systemic Sclerosis (SSc)**

A major autoimmune disease of connective tissues is Systemic sclerosis (SSc) where skin and internal organs manifest varied clinical features of heterogeneous nature having more prevalence in females than in males (21). The pathological outcome of systemic sclerosis is extensive skin, blood vessels, lungs and GI tract fibrosis (22). The researchers are of the view that anomalous production of IL-1a in SSc patient’s skin lesioned fibroblasts to induce IL-6 and procollagen which is having an epigenetic component that is causing fibrosis (23). Such a mechanism can operate at three different levels, i.e., gene transcriptional regulation, RNA stability control and degradation of collagen molecules before it secretes (24).

Earlier in 2013, a study published in *The Journal of Investigative Dermatology* reported the upregulation of p300, an acetyl-transferase having a predominant role in SSc and which is controlled by TGF-β (25). The etiology of SSc is reportedly connected to dysregulation in the epigenetic landscape of certain genes. DNA methylation at promotor region remains at the core of such research. Several important targets in this connection are reported. The hypomethylated genes in SSc are ITGA9,

COL23A, ADAM12, COL4A2 and MYO1E, including transcription factors such as RUNX1, RUNX2 and RUNX3 in both dSSc and ISSc (26). The hypermethylation in promotor region of Foxp3 in SSc patients is determined as one of the other factors in the development of SSc (27). The defective angiogenesis and micro vasculopathy in SSc patients is linked to lower expression of NRP1 that disturbs VEGF-A/VEGFR-2 (28). Recently, a methyl cap binding protein MeCP-2 has been shown to have a predominant role in systemic fibrosis (29). Mechanistically, TGF-B regulates expression of MeCP-2 in SSc fibroblasts, which regulates expression of extra cellular matrix that epigenetically represses sFRP-1 antagonist of Wnt signaling pathway. This in turn enhanced Wnt signaling, which favored fibrosis through glycolysis. MeCP-2 can be targeted as a key regulator in SSc fibrosis (29). Vasculopathy is one of the hallmarks of SSc and has been recently linked to trappin-2 which upregulates in absence of Fli1 to cause pathological conditions of vessels (30). Emerging data provides strong connection of the long noncoding RNA (NRIR) in driving the response of IFNs and its functional relevance in systemic sclerosis (31).

Several epigenetic therapies at present are considered to cure SSc, which targets including but not limited to histone modifications (32) and methylation regulation (29). Table:1 illustrates the autoimmune diseases, its epigenetic cause and targeted gene and cells.

**Table -1 Epigenetic Alterations in Autoimmune Diseases at the Level of DNA Methylation.**

Cell Type	DISEASES	GENES ALTERATION	EPIGENETIC ALTERATION (DNA)		PATHOLOGICAL SITE	REFERENCES
			Hyper methylation	Hypo methylation		
Peripheral blood CD4+ T cell	SLE, RA, SSc	CD40LG		√	CD40L (B cell costimulatory molecule encoded on the X chromosome)	(33)(34, 35)
	SLE, SSc, pSS	CD70		√	CD70, B cell costimulatory molecule associated with overproduction of IgG	(36-38)
	SSc, T1DM, RA	FOXP3	√		Forkhead box protein 3, involved in quantitative defects of regulatory T cells	(27, 39, 40)

	MS	HLA-DRB1		√	HLA class II beta chain	(41)
	SLE	IL10, IL13		√	Involved in autoantibody production and tissue damage	(42, 43)
	SLE	IRF5, IFIT2		√	Involved in type I interferon pathway	(44)
	SLE	ITGAL		√	Integrin $\alpha$ -L, associated with cell-cell adhesion	(45)
	SLE	PRF1		√	Perforin 1, involved in autoreactive killing	(46)
Naïve CD4+ T cell	SLE, pSS	STAT1, IFI44L, USP18		√	Involved in type I interferon pathway	(47, 48)
	pSS	LTA		√	Lymphotoxin- $\alpha$	(47)
	pSS	RUNX1	√		Transcription factor associated to lymphoma	(47)
B cell	SLE	CD5		√	CD5, involved in activation and expansion of autoreactive B cells	(49)
	SLE	HRES-1		√	Human endogenous retroviruses proteins, involved in induction of cross-reactive autoantibodies	(50)
PBMC	RA	IL-6		√	IL-6, involved in B cell response	(51)
	SLE	IFNGR2, MMP14		√	IFN- $\gamma$ receptor 1, Matrix metalloproteinase-14, involved in inflammation	(52)
	SLE	LCN2		√	Neutrophil gelatinase-associated lipocalin, iron transporter and marker for SLE	(52)
	MS	SHP-1	√		A negative regulator of cytokine signaling through NF- $\kappa$ B and STATs	(53)
CD14+ monocyte	T1DM	HLA-DQB1		√	HLA class II	(54)
	T1DM	RFXAP		√	HLA class II regulating element	(54)
	T1DM	NFKB1A		√	Regulator of apoptosis and inflammation	(54)
	T1DM	GAD2		√	GAD65, a major autoantigen involved in T1D	(54)
	T1DM	TNF	√		Key inflammatory cytokine	(54)
	T1DM	CD6	√		Involved in lymphocyte activation and differentiation	(54)
Fibroblast	SSc	FLI1	√		Involved in type I collagen expression	(55)
Fibroblast, PBMC	SSc	DKK1, SFRP1	√		Wnt signaling antagonists	(56)

Synovial fibroblast	RA	SFRP1, SFRP4	√		Wnt signaling antagonists	(57, 58)
	RA	DR3	√		Death receptor 3, associated with cell apoptosis	(59)
NAWM	MS	PAD2		√	Peptidyl arginine deiminase type II, responsible for the increased citrullinated myelin basic protein	(60)
LSG	pSS	DST	√		BP230, bullous pemphigoid antigen 1 protein	(61)

**\*Abbreviations:** SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; MS, multiple sclerosis; SSc, Systemic sclerosis; T1DM, type 1 diabetes; pSS, primary

Sjogren’s syndrome; PBMC, peripheral blood mononuclear cell; NAWM, normal appearing white matter; LSG, labial salivary gland.

**Table: 2 Epigenetic alterations in autoimmune diseases at the level of histone modification.**

Cell type	Disease	Epigenetic Alteration	Affected gene	Dysregulation		Reference s
				upregulation	downregulation	
T cell	SLE	H3 and H4 hypoacetylation, H3K9 hypomethylation	ND			(62)
		Increased histone H3 acetylation at lysine 18 (H3K18ac)	IL10	√		(29)
	Increased H3 acetylation, dimethylated H3 lysine4 (H3K4me2)	CD70	√		(63)	
	T1DM	Increased H3K9me2	CTLA4		√	(64)
B cell	SSc	H4 hyperacetylation, decreased HDAC2 and HDAC7; H3K9 hypomethylation, decreased SUV39H2 (member of HMT), increased JHDM2A (member of HDM)	ND			Wang et al.,2011
Monocyte	T1DM	Increased H3K9 acetylation (H3K9Ac)	HLA-DRB1, HLA-DQB1	√		(65)
	SLE	Global H4 hyperacetylation	IRF1, RFX1 and BLIMP1	√		(66)
Fibroblast	SSc	Increased H3 and H4 deacetylation	FLI1		√	(55)
		Inhibition of H3K27me3	FOSL2	√		(67)
Synovial fibroblast	RA	Increased zeste homologue 2 (EZH2, member of HMT)	SFRP1		√	(57)
		Increased sirtuin 1 (Sirt1, member of HDAC)	ND			(68)
Oligodendr ocyte	MS	Increased histone H3 deacetylation	ND			(69)

**\*Abbreviations:** SLE, systemic lupus erythematosus; T1DM, type 1 diabetes; SSc Systemic sclerosis; RA, rheumatoid arthritis; MS, multiple sclerosis; HMT, histone methyltransferases; HDAC, histone deacetylase; HDM, histone demethylase. \*ND, Not determined.

## 2. Epigenetics of inflammatory bowel disease

Inflammatory bowel disease (IBD) is a chronic condition, accompanying patients throughout their whole life. It has been widely accepted that IBD is caused by the dysregulation of cell/cell junction, causing defective barrier activity, resulting in enhanced severity of the disease (70). It has been classified into two major classes as inflammatory ulcerative colitis and Crohn's disease (71). Moreover, Crohn's disease affects the entire GI tract, whereas ulcerative colitis is only affecting large bowel (72). The recent studies are focused on investigating the role of tight junction dynamics and factors responsible for microtubule organization. One such factor identified is ACF7 (70), the ablation of which demonstrates poor wound healing and tight junction dynamics.

Other studies demonstrated the role of DNA methylation in the onset and prevalence of IBD influenced by the microbiome. The study by Harris et al. in 2016 revealed a close relationship between the epigenome of colon and microbiome in adolescents and children, demonstrating several differentially methylated regions having a number of possible therapeutic targets for treating IBD (73). Intestinal epithelial cell methylation and transcription patterns in children IBD patients define further subtypes and disease association (74). Another study demonstrates that a catalytic subunit PRC2, the EZH2 in epithelial cells, is responsible for keeping the epithelial cell barrier integrity (75).

Gene regulation is key factor in developing a certain phenotype, which is regulated by DNA methylation. Genome wide DNA methylation studies have the potential to provide therapeutic targets, which can be used to improve disease phenotypes. A whole genome DNA methylation study performed on the naïve ulcerative colitis patients determined

several regions of hypo- and hyper-methylated regions of genome that in future can be used as potential therapeutic targets in ulcerative colitis patients (76). There are 577 differential DNA methylation sites with 210 target genes shown in a study responsible for chronic inflammation of colon in epithelial cells (77). A cohort of 149 IBD patients PBMCs provide differential epigenetic regulation of genes and pathways including TRIM39-RPP2 and TRAF6 (78). Another important gene, Na<sup>+</sup>/H<sup>+</sup> exchanger-3 (NHE3), which is responsible for Na absorption and is associated with IBD, is regulated by epigenetic modulation of DNA methylation (79).

Microbiota diversity and its response is strongly related to IBD, and recently a mechanism by which it can interact is dissected. A study by Kelly et al. illustrated a new insight in H3K4me3 involved in key pathways of immunoglobulin, cell survival, metabolism and cell to cell signaling. They identified some unexplored key targets in epithelial cells which can be influenced by commensal microbiota in infant IBD patients (80). There are several genetic markers identified that are associated with IBD and IgG, i.e., IKZF1, LAMB1, and MGAT3. Recently, methylation in promotor region of MGAT3 in CD3<sup>+</sup> T cells from patients with ulcerative colitis has been detected (81). All such targets are providing an opportunity to look for further details of its involvement in disease prognosis and cure. The recent technologies of genome editing, including CRISPR-Cas9 (82), can be applied to rewrite the epigenome to its normal state, eliminating the drastic effects of environmental and other burdens from genome. It is important to keep full follow-up record of such experiments to eliminate the unwanted outcomes of such applications and to keep the safety concerns associated with each manipulation in mind and do compulsory experimentation before taking any drug or therapeutic agent for clinical trials.

## 3. The mechanistic insights into systemic lupus erythematosus

The immune system capability to recognize self from non-self is essential to conduct defense against foreign antigens (83) and is accomplished during the early developmental

stage. Females are more prone to autoimmune diseases than males (84), as is the case of systemic lupus erythematosus, a multisystem chronic autoimmune disease (85) resulting from T cell hypomethylation, because of lower expression and activity of DNMT1 in Lupus T cells (86), characterized by a defective ERK signaling pathway (87), which is reported nine times higher in females of reproductive age (84).

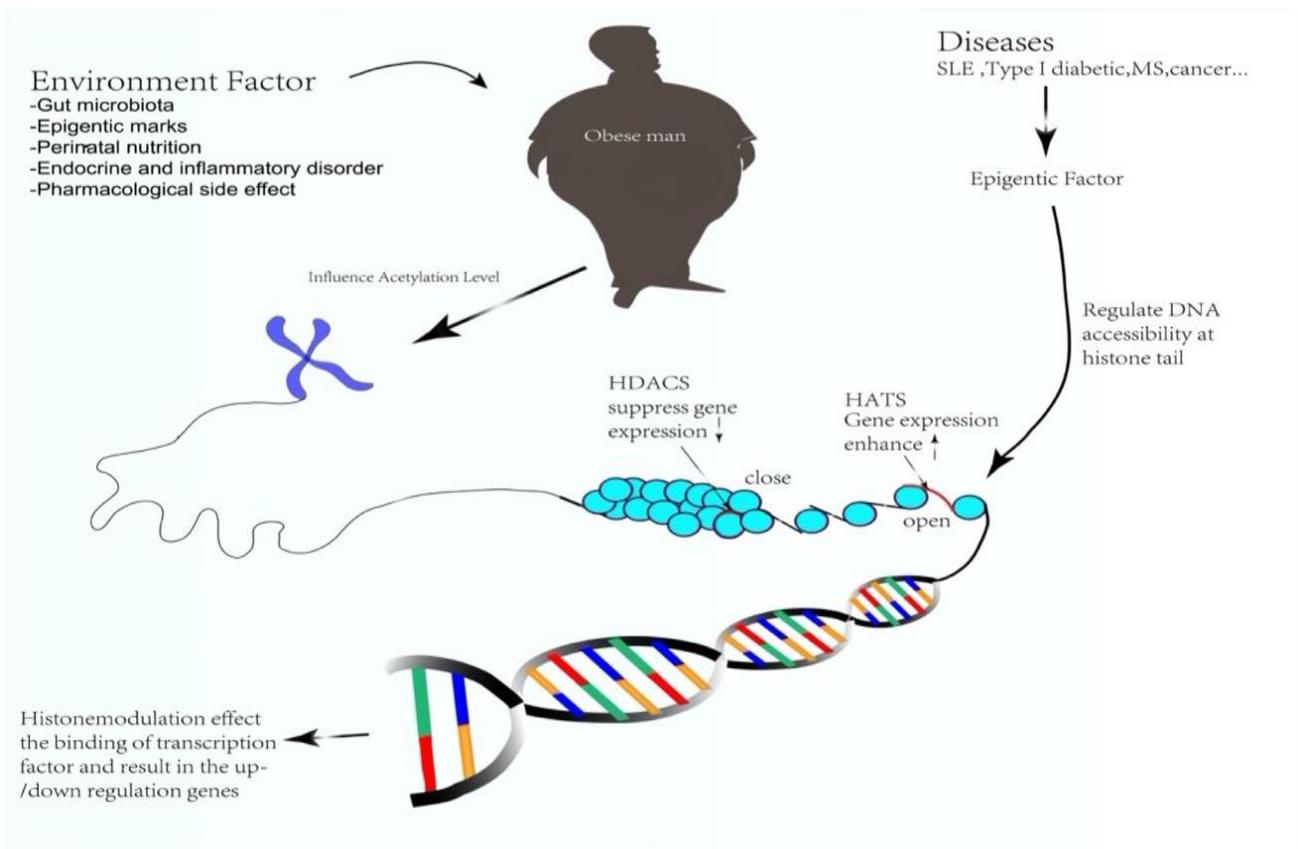
The predominant adaptive immune cells, B lymphocytes and T lymphocytes, are both involved in the development of SLE (83). Methylation of certain genes is one of the of the major reasons for the autoimmune diseases and it has been found that B cell promoter hyper-methylation is associated with SLE pathogenesis (88). CD4+T cells from SLE patients with lower expression of TNFAIP3, because of hyper-methylation of histone H3K4, resulting over production of pro-inflammatory cytokines, appears to be a probable cause of SLE (89). The TALEN mediated gene enhancer knock out of TNFAIP3 results in mimicking the phenotype of molecular SLE (6). Genome-wide association studies in SLE identified more than 50 risk gene or loci to higher heritability of SLE (90). Recent genome sequencing technologies have uncovered unprecedented information regarding the development of autoimmune diseases. The perturb-ATAC sequencing, for example, has shown enrichment of NF- $\kappa$ B binding sites near the causative variations of SLE and SSc (91) and these sites show altered accessibility at chromatin level when NFKB1 or RELA are depleted (92).

A shift in DNA methylation status in naïve CD4+ T cells was observed in favor of T cell activation in systemic lupus erythematosus patients with significant increase in EZH2 binding enrichment near high methylation activity in lupus patients (93). Furthermore, it was determined that EZH2 have a predominant role in T cell adhesion by upregulating JAM-A, hence providing a therapeutic target for lupus treatment by either blocking expression of EZH2 or JAM-A to limit T cell migration or adhesion (94). The lupus patients demonstrate demethylated CD4<sup>+</sup>CD28<sup>+</sup>KIR<sup>+</sup>CD11a<sup>hi</sup> T cells

characterized by epigenetic modulation, resulting in pro-inflammatory cytokines, providing another therapeutic avenue to treat lupus by either eliminating these cells or blocking them to produce pro-inflammatory cytokines (84).

#### 4. Mechanistic insight of protein acetylation and de-acetylation

Epigenetic modifications control the expression of genes that became the focus of basic and medical research to find suitable drug targets for prevention and cure of autoimmune diseases. Autoimmune diseases in particular are prone to histone acetylation and de-acetylation, which demands a detailed investigation of the mechanistic understanding of these modifications (95). The interaction of negatively charged DNA backbone is possible because of the positively charged histones N-terminal, which is neutralized by histone acetylation, hence weakening DNA binding and making the chromatin accessible to transcription (96). HATs and HDACs are the two enzyme classes responsible for acetylation and de-acetylation respectively. The chromatin is closed when de-acetylation takes place, consequently resulting in decreased gene expression due to no access by transcription factors. The environmental effects and diseases are leading to affect the expectancy and living quality of an individual as depicted in Figure 1. Currently, more than 2000 proteins are identified to be acetylated in mammalian cells which enhance the canvas of acetylation comparable to other major post translational mechanisms such as phosphorylation and ubiquitination (97). A wide range of proteins involved in different cellular processes undergo acetylation, making it one of the important events to be studied in detail. Almost all enzymes involved in gluconeogenesis, glycolysis, TCA cycle, urea cycle, fatty acid oxidation, and glycogen and nitrogen metabolism are acetylated (43). There are several mechanisms by which acetylation regulate the substrate availability, one of which is by blocking the enzyme binding to substrate; another is blocking the binding of metabolites. Acetylation can also modulate the localization of proteins (98).



**Figure - 1:** Environmental factors induce obesity and disease by affecting acetylation and de-acetylation of proteins

## 5. Conclusion remarks

In summary, epigenetic modifications play a crucial role in autoimmune disease onset, which if monitored and controlled in homeostasis by certain therapies and therapeutic agents can relieve many of the disease symptoms. DNA methylation and

protein acetylation play a very important role in maintaining the epigenome of organisms, hence more focused studies on the safety concerns of these targets are required to perform before bringing bench research to clinical settings.

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