

Acetylation of Histone and Modification of Gene Expression via HDAC Inhibitors Affects the Obesity

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Obesity is due to imbalance between energy intake and energy expenditure. Adipose tissues are the main site for the fat storage as well as for dissipation. There are two types of adipose tissues: white adipose tissue, which store fat as triglyceride, brown adipose tissue, which burns the fat into energy through the thermogenesis due to uncoupling protein1 present in inner mitochondrial membrane. Histone acylation causes changes in the chromatin structure without causing any change in the deoxyribonucleic acid sequence and thus regulate gene expression. Histone deacetylase causes the deacylation of histone and interfere with function of histone. Thus histone deacetylase inhibitors alter the expression of thermogenic gene encoding uncoupling protein 1, peroxisome proliferator activated receptor γ and also causes browning or beiging of white adipose tissue and increases the energy expenditure.

Keywords: Obesity, Adipose tissue, Thermogenesis, Gene, Histone deacetylase.

Obesity is abnormal or excessive fats accumulate in adipose tissue. The excess fat accumulates either in waist and trunk or in periphery of the body¹. Obesity can be explained on the basis of Body mass index (BMI), although another definition is based on waist circumference². According to classification based on BMI, normal healthy body weight is when BMI falls in between 18.5 kg/m² - 24.9 kg/m², overweight between 25 kg/m²- 29.9 kg/m² and obesity \geq 30 kg/m². The

obesity can be termed as severe when BMI is more than 40 kg/m², shows the maximum changes and is very difficult to treat without surgery³⁻⁵. As obesity is increasing day by day among the population worldwide, the incidence of metabolic disorders is also increasing along with this. Metabolic disorder is connected with a higher risk of a variety of diseases, including cardiovascular disease, insulin resistance, diabetes, hypertension and hyperlipidemia⁶. The increasing BMI can also

lead to incidences of several types of cancer^{7,8}. The health consequence of obesity increases the possibility of sudden or unexpected death and also leads to severe health conditions that may decrease the overall aspects of life. The use of energy rich, less nutrient foods with large amount of saturated fats and sugar, and also decreased physical activity can increase the obesity rates⁹. Excess fats are firstly stored in the adipose tissues and then in non adipose tissue. Adipose tissues are of two types: white adipose tissue and brown adipose tissue. White adipose tissues are the primary location to store the metabolic energy in the form of triglycerides. Whereas, brown adipose tissue which is rich in mitochondria, contains uncoupling protein 1 (UCP1), results in the conversion of energy into heat and regulate the energy expenditure. UCP1 is a fatty acid ion, which acts as a H⁺ symporter, which bypass the electron chemical gradient through the inner mitochondrial membrane with the help of ATP synthase and results in uncoupling of the mitochondrial oxidative phosphorylation and thereby transform the stored energy into heat^{10,11}. Studies had shown that the difference in percentage of obesity can be observed between genetically specific subpopulations, for example American Indians and Northern European people living in proportionate environments, which indicate that the obesity may be issues of gene and environment interactions. The mechanism through which the environmental factors and genes interact basically includes epigenetic factors for example deoxyribonucleic acid (DNA) methylation and histone modifications¹². Epigenesis includes histone acetylation and also without changing the actual DNA structure, epigenesis changes the structure of chromatin and thus regulates the gene expression¹³. The DNA molecule is wrapped around the histone octamer to form the basic structure of chromatin called nucleosome. Transcriptional co-activators show basic histone acetyltransferase action and acetylate the histone. The acetylated histone thus activates other co-activators and chromatin modifying enzymes. This results in alteration of the chromatin structure and activate RNA polymerase II, which is then followed by gene transcription¹⁴. Histone acetylation is basically maintained through the action of histone acetyltransferases (HATs)^{13,15}. Obesity-associated genes may change the encoded

amino acid pattern, and also the mass and action of translated proteins, changes may also be related to the eating patterns that affect individual sensitivity to gain weight in specific environment¹⁶. Histone or lysine deacetylase (HDAC) enzyme mainly act on histone and non histone proteins, which remove acetyl and acyl group from their lysine residue to regulate various transcription, cell death and metabolism¹⁷. Deacetylation of histone through HDAC results in heterochromatin formation and gene repression^{18,19}.

Classification of HDAC

Histone deacetylase family is classified into four major classes, along with the members:

1. Class I HDAC (include HDAC 1, 2, 3, 8),
2. Class II HDAC (include HDAC 4, 5, 6, 7, 9, 10),
3. Class III HDAC (include SIRT 1-7),
4. Class IV HDAC (include HDAC 11).

Class I, Class II, Class IV HDAC's members are Zn²⁺ dependant deacetylases, which catalyzes the removal of acetyl group from lysine residue in histone and other cellular proteins, whereas Class III HDAC members are NAD⁺ dependant deacetylase^{13,20}.

Class I HDAC

Class I HDAC has sequel related to the yeast Rpd3 protein and also having a nuclear localization signal (NLS), although it does not contain nuclear export signal. Therefore these HDAC are mainly localized in nucleus only²¹. This shows high enzymatic activity towards histone substrate. Structurally Class I HDAC comprises of conserved deacetylase site plus short amino and carboxy terminal extensions. Comparative to other members HDAC1 and HDAC2 are almost similar to each other and present along with each other in some complexes such as Sin3 (transcriptional regulatory protein), NuRD (nucleosome remodeling deacetylase), CoREST, and PRC2 (polycomb repressive complex 2). HDAC3 is present in different complexes such as N-CoR-SMRT complex. Also, there is no distinguished complex for HDAC8^{22,23}. Studies had shown that HDAC1 is a prominent target in regulating the thermogenic action in brown adipose tissue. Studies had also shown that Class I and Class II HDAC inhibitors show effect on thermogenic gene expressions of brown fat²⁴. Studies had shown that the effect of β adrenergic receptor activation on the

chromatin state of brown adipocytes. β adrenergic receptor stimulates UCP1 expression and result in acetylation of histone. Studies had investigated that this effect can be seen with down regulation of Class I HDAC specially HDAC3. HDAC3 inhibitor increases the UCP1 expression and cause histone acetylation. Isoproterenol act by down regulating the HDAC3 and increases the UCP1 expression²⁵. Li FenFen et al had studied that HDAC1 regulate the H3K27 deacetylation and methylation and due to which HDAC1 negatively regulates the gene expressions of brown adipocytes. Thus by inhibiting the HDAC1 enhances the thermogenic expression of brown adipocytes. Study revealed that by inhibiting the HDAC1 may be the novel target for the treatment of obesity and by enhancing the brown adipocytes thermogenesis and energy expenditure²⁴.

Class II HDAC

Class II HDAC contain extra regulatory domain as compared to Class I HDAC, therefore are larger protein than Class I HDACs. Class II HDAC contains nuclear localization signal as well as nuclear export signal and travel between cytoplasm and nucleus which depends on phosphorylation. Class II HDAC again classified in to two more sub category: Class IIa HDAC, which include HDAC4, HDAC5, HDAC7 and HDAC9, Class IIb, which include HDAC6, HDAC10^{21,26,27}.

In Class II a, HDAC4 is present in brain and growth plates of skeleton, HDAC 5 and HDAC 9 are present in muscle, brain and heart, and HDAC 7 is found in endothelial cells and thymocyte. Class II a HDAC members contains N-terminal extensions, which also contains the binding site for the transcription factor myocyte enhancer factor 2 (MEF2) and also for the chaperone protein 14-3-3, and make the HDACs signal responsive. Various kinases (calcium/calmodulin-dependent protein kinase (CaMK) and protein kinase D (PKD) phosphorylate the HDACs. These phosphorylated HDAC bind to the 14-3-3 protein, after which the complex travel from the nucleus to the cytoplasm. The phosphorylation of Class IIa HDACs renders a mechanism for connecting extracellular signals with transcription and shows its action in diverse tissues during production and disease^{22,28,29}.

Class II b HDAC include only HDAC6, HDAC10. HDAC 6 is the main deacetylase enzyme

present in cytoplasm of mammals. HDAC6 is a unique enzyme which basically contain two catalytic/ deacetylase domain and zinc finger motif (HUB) site at the C-terminus. HDAC mainly deacetylates the cytoskeleton proteins for example α -tubulin and contractin and also transmembrane proteins for example the interferon receptor (IFN α R) and chaperones. HDAC10 is closely related to HDAC6. HDAC10 has its catalytic site on its N-terminal (22,30). Studies had shown that HDAC5 is an important component of leptin signal and is found in hypothalamus of brain. Hence HDAC5 play important role in food intake as well as body weight (31). HDAC6 is mainly located in the cytoplasm and deacetylates different cytoplasmic substrates for example tubulin, Hsp90, cortactin, and MFN1. Study had identified that HDAC6 is a key adjuster of mitochondrial thermogenesis, which is related to the lipid metabolism in brown adipose tissue via cAMP-Pka signaling. Inhibition of HDAC6 decreases the cellular cAMP, which is a key adjuster of uncoupling protein1 (UCP1), which in results causes building up of fats in brown adipose tissue (32). Studies had shown that deletion of HDAC9 results in reduction of BMI and modified metabolic homeostasis, by preventing harmful actions of ingested chronic high-fat food on adipogenic differentiation, enhances the adiponectin responses, and via enhancing the beige adipogenesis, this increases the energy expenditure²⁰

Class III HDAC

Class III HDAC is only class of HDAC which comprises of nicotinamide adenine dinucleotide (NAD)-dependent deacetylases, having sequel which is related to the yeast Sir2 protein. Hence, called as Sirtuins (SIRTs). SIRTs act on vast maximum of cellular proteins present in the nucleus, cytoplasm and mitochondria for post-translational alteration by acetylation or ADP-ribosylation. Out of all SIRTs, SIRT1 and SIRT2 travel among the nucleus and cytoplasm. SIRT, through NAD⁺ hydrolysis, also represents the deacetylation of protein and combine cellular energy and redox elements to produce several signaling and outlast mechanisms that control various transcription factors for example NF- κ B, p53 and forkheadbox (FOX) proteins. SIRT1 also show its role in tumor initiation. SIRT2 is

present in cytoplasm and deacetylase α -tubulin protein. SIRT3, SIRT4, SIRT5 are present in mitochondria. SIRT3 regulate the actions of acetyl coenzyme A synthetase 2 and deacetylating complex I of the respiratory chain involved in ATP synthesis and play a role to maintain the energetic cell homeostasis. SIRT4 shows ADP ribosylase action that deactivates the glutamate dehydrogenase and also hinder the insulin secretion in β cells of pancreas. SIRT5 increases the ammonia detoxification through deacetylation and stimulation of carbamoyl phosphate synthetase 1 to increase the ammonia detoxification. SIRT6 and 7 are mainly present in the nucleus where they show a key role in DNA repair and ribosomal RNA transcription, respectively^{21,30}. Activation of SIRT1 increases the functions of mitochondrial, increases the expenditure of energy, also increases the sensitivity of insulin and also decreases the inflammation³³. SIRT1 results in deacylation of PPAR γ , which is a master regulator of adipogenesis. SIRT also increases the binding of deacylated PPAR γ with (PRDM 16) and promote the conversion of white adipose tissue to beige phenotype. PRDM16 is a co-regulator of PGC1 α and increases the UCP1 expressions. Also PRDM16 regulates the adipogenesis of brown adipocytes and also brown adipose tissue functions. Thus SIRT by deacylating PPAR γ play a role in obesity³⁴. SIRT2, via deacetylation and activation of forkhead box protein O1, promotes the adipogenesis and lipolysis, whereas SIRT4, via deacetylation and inhibition of malonyl-coenzyme A decarboxylase activity, decreases the lipid oxidation. SIRT3 also shows mitochondrial oxidation and fatty acid oxidation and by showing protein deacetylation. SIRT3 increases mitochondrial antioxidative capabilities. SIRT1 and SIRT3 collectively promote the mitochondrial unfolded protein response (UPR), which further act by protecting mitochondria from proteotoxic stress caused by misfolded proteins that increases mitochondrial protein quality. SIRT5 increases the fatty acid oxidation, mitochondrial respiration and also control lysine acylation of mitochondrial proteins. So, above all the SIRT family shows function in regulating mitochondrial function and metabolism³³.

Class IV HDAC

HDAC11 is the only member of Class IV

HDAC. HDAC11 is present in nucleus. Studies had also shown that HDAC11 consists of long chain fatty acid deacetylase. HDAC11 contains a catalytic site in the N-terminal region. HDAC11 is only member of Class IV HDAC and this HDAC11 is found in heart, brain, muscles, kidney and testes. HDAC11 regulates the equilibrium among immune tolerance and immune activation in CD 4+ and T-cells (20–22). Study had shown that HDAC 11 reduces the brown adipose tissue thermogenic gene expressions and also prevent being of white adipose tissue. Study had shown that the HDAC11 associated with bromodomain and extraterminal (BET) family member BRD2, acts as inhibitor of brown adipose tissue differentiation and thermogenic gene expression. The deletion of HDAC11 causes the enhanced brown adipose tissue uncoupling protein 1 expression, enhanced being of white adipose tissue, and also increases the thermogenic gene expression in return to β 3 adrenergic signal. Therefore the study provides finding to the possibility of the selective HDAC11 inhibitor can develop to enhance the expenditure of energy for obesity treatment and other metabolic disorders³⁵.

Role and mechanism of HDAC inhibitor in obesity: HDAC inhibitors are considered as powerful therapeutics agents for the treatment of cardiovascular disease, obesity and diabetes³⁶. Various studies in previous years evaluated the actions of different types of inhibitors of HDAC in regulating the gene expressions of brown fat¹³.

HDAC inhibitors represent the different types of chemotherapeutic agents that interrupt the functions of HDAC through indirect stimulation of histone acetylation, HDAC inhibitors also modify the gene expression. HDAC inhibitors can be obtained by natural as well as synthetic sources³⁰. Structurally HDAC inhibitors consists of zinc binding site, surface binding site and a link that connect these two binding sites along with the hydrophobic catalytic site channels. HDAC inhibitors mainly bind on the zinc ion on the catalytic site of enzyme and inhibit the activity of HDAC^{37,38}.

HDAC inhibitors can be classified into various categories

1. Hydroxamate: Trichostatin A (TSA), Suberoylanilide hydroxamic acid (SAHA)

(Vorinostat), LAQ824, LBH589 (Panabinstat), or PXD101 (Belinostat), M344, CR2408, abexinostat hydrochloride (PCI-24781)

2. Aliphatic acid: Sodium butyrate (NaB), Valproic acid (VPA), Phenylbutyric acid

3. Benzamides: MS-275 (Entinostat)

4. Tetrapeptide: Apicidin, Romidepsin, Trapoxin B

5. Sirtuin inhibitor (SIRTi):

a) Pan inhibitor: Nicotinamide

b) Specific (SIRT1 & SIRT2) inhibitor: Sirtinol, Cambinol, EX-527 (30,37).

Hydroxamates: Hydroxamates are the most commonly used HDAC inhibitors and have very high binding affinity with the zinc ion. The first HDAC inhibitors approved by the FDA was Suberoylanilide hydroxamic acid (SAHA), which shows its inhibitory action as zinc binding group and thus inhibit the deacylation by HDAC. Trichostatin A (TSA) is a naturally derived compound which shows the highest HDAC inhibition activity. Hydroxamates have ability to inhibit all types of HDAC as compared to other HDAC inhibitors. Various advantages of hydroxamates are: higher zinc binding ability, good solubility, can be easily synthesized. As these hydroxamates can bind to other zinc dependant enzymes such as aminopeptidases, metalloproteinases and carbonic anhydrase, which lead produce undesirable effects^{37,38}.

Recent studies had shown that hydroxamates for example trichostatin A (TSA) inhibit the gene expressions of uncoupling protein 1, peroxisome proliferator activated receptor γ , PR domain-containing protein 16 (prdm-16). TSA also act through the stimulation of Pgc1 α expressions in brown adipose tissue I brown adipocyte. TSA act dose dependently to show the action. Suberoylanilide hydroxamic acid (SAHA) also act dose dependently, and act the same way as TSA. TSA and SAHA show their inhibitory action on both Class I HDAC and Class II HDAC. Study represent that TSA and SAHA show their maximum inhibitory action at dose 500 η M and 5 η M respectively^{13,39}.

Aliphatic acids: Aliphatic acids or mainly carboxylic acid derivative have less zinc ion binding affinity as compared to hydroxamates or benzamide. Aliphatic acid with short chain fatty acids, such as valproic acid, butyric acid/ sodium butyrate, phenylbutyric acid/ phenylbutyrate shows

HDAC inhibitory action. These aliphatic acids HDAC inhibitor inhibits mainly Class I HDAC and Class IIa HDAC^{37,38}.

Valproic acid is widely used as anti epileptic drug. But most recently it can be used as HDAC inhibitor. Valproic acid, which is a short chain fatty acid, also shows HDAC inhibition activity. However it was previously suggested that valproic acid induces the weight gain. But, recently study had shown that Valproic acid suppresses adipogenesis. Valproic acid and other HDAC inhibitors are mainly involved in the improvement of growth and differentiation of adipocytes by activating the adenosine monophosphate activated protein kinase, which is a controller mechanism of cellular metabolism. Study had shown that valproic acid acts via inhibiting fatty acid synthase (FAS) enzyme. Fatty acid synthase plays a major role in lipogenesis in mammals and also catalyses the long chain fatty acid synthesis from acetyl co-enzyme A and malonyl co-enzyme A. FAS activity is regulated by the various hormones and nutrients as well. Enhanced FAS gene expressions in adipose tissue results in accumulation of visceral fat and also changes the insulin sensitivity in humans. Therefore FAS is the major target for the obesity and valproic acid causes the down regulation of the fatty acid synthase enzyme and thus reduces the lipid accumulation in human adipose tissue. Valproic acid also reduces the Pparg and Cebpa gene expression and regulate the expressions of lipolytic genes such as Atgl, Hsl and Mgl, as well as the adipogenic genes such as Fabp4 (fatty acid binding protein 4), Lpl (lipoprotein lipase) and Cd36, a transporter of long-chain fatty acids. Study also showed that valproic acid also inhibit USF-1 gene, as polymorphism of USF-1 genes is associated with the hyperlipidemia and obesity^{40,41}.

Benzamides: Benzamides such as MS-275 also known as Entinostat, inhibits some of Class I HDAC. The amino group present in benzamide form chelate with zinc ion and therefore show its inhibitory action^{37,38}.

Studies had shown that benzamide MS-275 acts dose dependently and stimulate the thermogenic gene expressions of brown fat, which include uncoupling protein 1, peroxisome proliferator activated receptor γ , fatty acid elongase 3 (Elovl3) and peroxisome proliferator activated

receptor gamma coactivator 1 alpha (Pgc1 α). Studies showed that the treatment of HIB-1b cell line with benzamide MS-275 significantly increases the basal and isoproterenol stimulated gene expressions of brown fat including uncoupling protein 1, peroxisome proliferator activated receptor gamma coactivator 1 alpha, peroxisome proliferator activated receptor gamma coactivator 1 β , acyl- CoA oxidase1 (ACOX 1), cell death inducing DFFA like effector A (CIDEA), and peroxisome proliferator activated receptor and study also show that MS- 275 do not any effect on COX-1 expressions. The study also showed that along with the stimulating brown adipocyte thermogenic regulations, this MS-275 treatment also regulate enhanced uncoupling protein 1 messenger RNA expressions in 3T3-L1 cell line of white adipocytes. Ucp1 is a mitochondrial uncoupling agent in brown adipose tissues and Pgc1 α play as a master controller of brown fat thermogenesis^{13,39,42}.

MS-275 enhances the oxidative metabolism in adipose tissue and also in muscle tissues, thus increases the energy expenditure, which can lead to the reduction in body weight. Study had shown that MS-275 inhibit action of both HDAC1 and HDAC3. Study also showed that animal reduces 10% of body weight via treatment of MS-275⁴³.

Tetrapeptide/Depsipeptide: Apicidin, romidepsin, trapoxin B are the example of tetrapeptide/ depsipeptide. Apicidin is a cyclic tetrapeptide which is isolated from the fungus *Fusarium Spp.* Apicidin is known as selective inhibitor of HDAC class I. Apicidin bind to HDAC class I and interfere with the deacetylation of protein^{44,45}.

Romidepsin is another tetrapeptide/ depsipeptide which inhibits HDAC1 and HDAC2 and is widely used as anti cancer drug. Recent study had shown that romidepsin, via the stimulation of adipocytes linked with transcriptional factors which is required for the adipocytes differentiation for example proliferator-activated gamma receptor (PPAR γ 2) and other genes related to adipogenesis including AD1, POQ, apetala 2 (AP2), phosphoenolpyruvate carboxykinase 1 (PCK1), lipoprotein lipase (LPL), lipase E (LIPE), induces the adipocytic differentiation. Expression of these genes can lead to obesity and diabetes^{46,47}.

Sirtuin inhibitor (SIRTi): Nicotinamide is the most widely used SIRT inhibitor, which inhibits the deacetylation of class III HDAC. Nicotinamide is form of vitamin B3 or amide form of niacin, which play important role in production of nicotinamide adenine dinucleotide (NAD⁺) and nicotinamide adenine dinucleotide phosphate (NADP⁺), which also takes part in biological processes such as energy production, redox reactions and biological rhythm. Nicotinamide deacetylates the SIRT1 and results in modification of various target protein actions and help the cell to survive in various stress conditions. Study had shown that circulating nicotinamide in form of nicotinamide phosphoribosyltransferase is related to obesity, insulin resistance, lipid profile and inflammation. Study had shown that there is increased expression of adipose tissue towards nicotinamide phosphoribosyl transferase, which results in induction of obesity (48–50). Study had shown that the nicotinamide can be considered as novel marker of systemic inflammation which is associated with fat depot, in the elderly population⁵⁰.

Another drug which is used as SIRT inhibitor is EX-527. Study had shown that EX-527 inhibits the SIRT1 and thus reduces the SIRT-1 phosphorylation and PRDM-16 deacetylation³⁴.

CONCLUSION

The study explains the different classes of Histone deacetylase as Class I, Class II, Class III and Class IV and their mechanism by which they play role in obesity. Class I, Class II and Class IV HDAC are Zn⁺ dependant deacetylase and by binding on the Zn⁺ they increases the incidence of obesity, whereas Class III HDAC, alternatively known as SIRT, is NAD⁺ dependant and reduces the incidence of obesity. In this study, different HDAC inhibitors were also explained. Out of which HDAC inhibitor hydroxamate, was the first approved HDAC inhibitor, aliphatic acids and benzamides act by binding on Zn⁺ and increases the gene expression required for acylation of histone protein and thus by increasing the gene expression these may lead to beiging or browning of white adipose tissue as well as energy expenditure. Other class of HDAC inhibitor which includes SIRT inhibitor or specifically Class

III HDAC inhibitor shows opposite effect than other classes of HDAC inhibitor. SIRT inhibitor interferes with NAD⁺ synthesis. Nicotinamide is example of SIRT inhibitor which increases the lipid profile as well as fat depot and thus induces obesity and inflammation, whereas Resveratrol, a polyphenolic compound, act by stimulating SIRT and show pharmacological properties in cardiovascular disease, obesity, aging and also in diabetes mellitus.

Conflict of Interest

There are no conflicts of interest.

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