Role of histone deacetylase 9 in regulating adipogenic differentiation and high fat diet-induced metabolic disease

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Adipose tissue serves as both a storage site for excess calories and as an endocrine organ, secreting hormones such as adiponectin that promote metabolic homeostasis. In obesity, adipose tissue expands primarily by hypertrophy (enlargement of existing adipocytes) rather than hyperplasia (generation of new adipocytes via adipogenic differentiation of preadipocytes). Progressive adipocyte hypertrophy leads to inflammation, insulin resistance, dyslipidemia, and ectopic lipid deposition, the hallmark characteristics of metabolic disease. We demonstrate that during chronic high fat feeding in mice, adipogenic differentiation is impaired due to the actions of histone deacetylase 9 (HDAC9), a member of the class II family of HDACs. Mechanistically, upregulated HDAC9 expression blocks the adipogenic differentiation program during chronic high fat feeding, leading to accumulation of improperly differentiated adipocytes with diminished expression of adiponectin. These adipocytes are inefficient at storing lipid, resulting in ectopic lipid deposition in the liver. HDAC9 gene deletion prevents the detrimental effects of chronic high fat feeding on adipogenic differentiation, increases adiponectin expression, and enhances energy expenditure by promoting beige adipogenesis, thus leading to reduced body mass and improved metabolic homeostasis. HDAC9 is therefore emerging as a critical regulator of adipose tissue health and a novel therapeutic target for obesity-related disease.

Introduction

Adipose tissue plays a fundamental metabolic role to store dietary calories as triglyceride lipid within the adipocytes, the major cellular component of the adipose tissue. Adipocytes also function as endocrine cells to produce various hormones (known as “adipokines”) that regulate whole-body insulin sensitivity, systemic glucose tolerance, and other metabolic activities. The number of adipocytes in humans is maintained at a remarkably constant level throughout adult life, despite the fact that adipocytes are constantly turning over.1-6 Since adipocytes are terminally differentiated, they are replenished by adipogenic precursor cells, termed preadipocytes, which are contained within adipose depots and differentiate into mature adipocytes in response to metabolic cues. Conversion of preadipocytes to adipocytes (referred to as “adipogenic differentiation” or “adipogenesis”) is a carefully regulated cellular event, tightly linked to metabolic activities at the whole-body level, and orchestrated at the cellular level by the actions of a number of transcription factors, including C/EBPα and PPARγ.

Adipogenesis is Constrained during Obesity

To accommodate excess dietary calories, adipose tissue can expand by hyperplasia (increasing adipocyte numbers through adipogenic differentiation) and/or hypertrophy (increasing the size of...
it is not clear why adipogenic differentiation is constrained during chronic caloric excess. Adipose tissue contains a huge reserve of precursor cells (adipose-derived stem cells and committed preadipocytes)—representing approximately 20–40% of the adipose tissue cellularity to support continuous remodeling of this tissue. It is estimated that daily turn-over of preadipocytes and adipocytes could be as high as 5% under some conditions, and obesity markedly stimulates preadipocyte replication. This does not, however, translate into generation of sufficient numbers of mature, functionally competent adipocytes to store the excess calories efficiently. Indeed, adipocytes that accumulate in obese adipose tissues are inadequately differentiated, exhibiting markedly reduced levels of adipogenic differentiation-specific genes. Defining the causes and consequences of constrained adipogenic differentiation in obesity is a matter of great interest in obesity research. We recently demonstrated that preadipocytes isolated from high fat diet-induced obese mice exhibit impaired adipogenic differentiation in vitro, suggesting that differentiation is constrained by factors endogenous to the cells themselves rather than the external environmental. The fact that impaired differentiation persists in vitro, even after several passages in culture, implies an epigenetic mechanism as the probable cause of impaired adipogenic differentiation in high fat diet-induced obesity.

![Figure 1](image-url)

Figure 1. Schematic diagram showing adipose tissue dynamics in lean and obese states. Mesenchymal stem cells generate committed preadipocytes that differentiate into unilocular white and multilocular beige (with increased mitochondrial content) adipocytes in response to adipogenic signals to maintain metabolic homeostasis. Chronic high fat diet (HFD)-induced obesity impairs adipogenesis, promotes enlargement of individual adipocytes, decreases beige adipocyte abundance, and increases adipose tissue inflammatory cells, leading to insulin resistance and other consequences of metabolic disease. Decline in histone deacetylase 9 (HDAC9) level precedes activation of the differentiation program, while elevated HDAC9 levels in HFD fed condition impairs activation of this program, promoting adipocyte hypertrophy.

**Histone Deacetylase 9 (HDAC9) is an Epigenetic Regulator of Adipogenesis**

Epigenetic processes introduce specific alterations in the chromatin structure which persist through cell divisions, allowing stable changes in gene expression that impart “memory” to the cells. To begin to understand how adipogenic differentiation is epigenetically disrupted by diet-induced obesity, we turned our attention to understanding the epigenetic mechanisms that control physiologic adipogenic differentiation in lean mice. Our investigations have focused on the chromatin histone modifying enzyme HDAC9, whose dynamic pattern of expression during adipogenic differentiation stands out among all of the other members of the HDAC/histone acetyltransferase family of genes. In lean states, HDAC9 is expressed at high levels in preadipocytes, and its level abruptly falls at the onset of adipogenic differentiation in both humans and mice. Elevating HDAC9 levels by transient transfection prevents adipogenic differentiation, while genetic ablation of HDAC9 accelerates this process, documenting that HDAC9 negatively regulates adipogenic differentiation. HDAC9 gene deletion, however, does not spontaneously activate adipogenic differentiation, suggesting that the abrupt fall in HDAC9 levels is required to prime responsiveness to adipogenic signals. The mechanisms whereby HDAC9 inhibits adipogenic differentiation are not fully elucidated. Current evidence suggests that HDAC9 blocks the action of key transcription factors to commence the adipogenic differentiation program. This effect appears to be related to interactions between HDAC9 and transcriptional repressor proteins in the cell nucleus, independent of HDAC9’s enzymatic activity. Indeed, as a class II HDAC, HDAC9 possesses very weak histone deacetylase enzymatic activity. These findings are consistent with a growing body of data implicating deacetylase-independent actions of many HDAC family members.

We subsequently determined that HDAC9 is a nutrient sensitive gene whose expression is upregulated in adipocytes from chronically high fat fed, obese...
Under these conditions, HDAC9 levels fail to decline in preadipocytes during in vitro adipogenic differentiation, suggesting that the regulatory mechanism controlling HDAC9 expression is disrupted in diet-induced obesity. Consequently, the adipogenic differentiation program is blocked, leading to generation of improperly differentiated adipocytes with impaired endocrine function and blunted lipid storage capacity. Importantly, HDAC9 gene deletion removes this block, allowing appropriate activation of the adipogenic differentiation program in adipocytes during chronic high fat feeding. This supports a role for HDAC9 as a molecular mediator of impaired adipogenic differentiation in obesity. How HDAC9 expression is regulated during adipogenic differentiation in lean mice, and how this process is pathologically disrupted in obesity, remain to be elucidated.

**HDAC9 Gene Deletion Protects Mice against Obesity-Related Metabolic Disease**

Based on the observations that upregulated HDAC9 expression constrains adipogenic differentiation in obesity, we postulated that HDAC9 gene deletion would protect against obesity-related metabolic disease by generating more functionally competent adipocytes capable of storing lipid, thus distributing the excess calories into a larger number of adipocytes and reducing the extent of adipocyte hypertrophy leading to cellular stress. Indeed, during chronic high fat diet, HDAC9 knockout mice exhibit smaller adipocytes, increased insulin sensitivity, enhanced glucose tolerance, and reduced hepatic lipid accumulation compared with wild-type mice despite similar food consumption and locomotor activities.

**HDAC9 Knockout Mice Exhibit Increased Energy Expenditure and Enhanced Adaptive Thermogenesis**

In addition to their improved metabolic state, HDAC9 knockout mice exhibit lower body weight and smaller adipose tissue mass compared with wild-type mice, both under chow and high fat fed conditions. This phenotype was not explained by differences in nutrient absorption between the two strains of mice (data not shown). This suggests an additional mechanism whereby HDAC9 knockout mice are protected against obesity-related disease: enhanced combustion of surplus dietary calories to produce dissipating heat energy. In support of this notion, HDAC9 knockout mice exhibit increased oxygen consumption, elevated energy expenditure, and enhanced adaptive thermogenesis.

Traditionally, adipocytes are classified as energy storing white adipocytes and energy combusting brown adipocytes, the latter of which turn dietary calories into dissipating heat energy. Our investigations, however, revealed that brown adipose tissue in the HDAC9 knockout mice, although phenotypically indistinguishable from wild-type mice, is actually reduced in mass, suggesting that increased calorie burning capacity of HDAC9 knockout mice is not related to differences in their brown adipose tissue content. We therefore sought an alternate explanation for the increased energy expenditure in HDAC9 knockout mice.

**HDAC9 Gene Deletion Augments “Beige” Adipocytes during Obesity**

In addition to white and brown adipocytes, a third class of adipocytes has recently been identified in subcutaneous adipose tissues of both mice and humans, which, like brown adipocytes, also turn dietary calories to dissipating heat energy. The abundance of this new class of adipocytes, referred hereafter as “beige” adipocytes, is inversely related to obesity and insulin resistance in humans, suggesting a potentially important role for beige adipocytes in modulating obesity and its consequences. Consistent with the human studies, we found that beige adipocyte abundance is reduced in chronically high fat fed obese wild-type mice. Interestingly, we observed that HDAC9 gene deletion increases beige adipocyte abundance in subcutaneous adipose tissues of lean mice, as evidenced by increased appearance of multicellular adipocytes (Fig. 2C), and expression of beige-specific genes PRDM16, UCP1, CIDEA, and PGC1α. Moreover, HDAC9 gene deletion prevented the reduction in the beige adipocyte gene expression during chronic high fat diet-induced obesity.

These findings likely help to explain the observations of elevated energy expenditure, increased oxygen consumption, and enhanced adaptive thermogenesis in HDAC9 knockout mice. Enhanced beige adipocytes in HDAC9 knockout mice may also cause and/or compensate for the loss of brown adipose tissue mass in these mice. How HDAC9 gene deletion increases beige adipocyte abundance is unknown; nor it is known how beige and brown adipose tissues cross-talk with each other to maintain metabolic energy homeostasis in mice. Brown adipose tissue loss was reported to induce a compensatory increase in beige adipocytes by altering sympathetic innervation of the white adipose tissues. Indeed, pharmacological activation of β3 adrenergic receptors augments beige phenotypic changes of white adipose tissue. These adipogenic precursor cells also undergo β-adrenergic receptor-independent activation of their beige adipogenic differentiation program, pointing to involvement of potentially multiple mechanisms. Identification of HDAC9, a nutrient sensitive gene, as a critical regulator of the calorie burning beige adipogenic program provides molecular insight how dietary caloric intake regulates caloric consumption pathways.

**FGF21**

FGF21, a member of the FGF family of proteins, functions as an endocrine hormone and exhibits therapeutic potential as an anti-obesity and anti-diabetic agent in preclinical studies. In addition to liver, FGF21 is secreted by the adipocytes, where it acts as a local autocrine/paracrine factor to enhance beige adipogenesis, presumably through a PGC1α-
FGF21 stimulates thermogenic gene expression in white adipose tissue, supporting adaptive thermogenesis, fatty acid oxidation, and lipid metabolism. We demonstrated significant FGF21 expression in subcutaneous, but not in visceral, adipose tissues of lean mice, which was markedly blunted in the setting of chronic high fat diet-induced obesity, indicating that FGF21 is a nutrient sensitive gene. While HDAC9 gene deletion did not affect FGF21 expression in lean mice, it completely prevented the reduction in FGF21 expression in obese mice. How chronic high fat diet-induced obesity reduces FGF21 levels in subcutaneous adipose tissue is not known, nor is it known how HDAC9 gene deletion reverses this process. FGF21 is expressed in beige adipocytes, so it is possible that the reduction in FGF21 levels during obesity could be consequent to reduced beige adipocyte abundance. In such case, HDAC9 could indirectly regulate FGF21 levels in subcutaneous adipose tissue through its influence on beige adipogenesis. Future investigations with sorted cells from subcutaneous adipose depots are required to demonstrate whether FGF21 is expressed exclusively by the beige adipocytes to act in autocrine fashion, or whether it is expressed by other cells in the adipose depot.

**HDAC9 as a Potential Therapeutic Target in Obesity: Present Understanding and Future Perspectives**

HDAC9 is emerging as an important regulatory molecule capable of profoundly influencing adipose tissue biology and metabolic homeostasis. The marked upregulation of HDAC9 expression in adipose tissues during chronic high fat diet-induced obesity clearly contributes to impaired adipogenic differentiation, as evidenced by our findings in HDAC9 knockout mice. It appears that HDAC9 gene deletion improves metabolic state not only through its favorable effects on adipogenic differentiation, but also by upregulating beige adipogenesis. Thus, inhibiting the actions of HDAC9 may favorably regulate both the balance between adipocyte hypertrophy and hyperplasia and the balance between energy storage and consumption in obesity. This dual effect potentially sets HDAC9 apart from many other potential therapeutic targets for obesity-related metabolic disease.

How could one potentially therapeutically target HDAC9 in obesity? First, it is important to point out that non-selective or selective HDAC enzymatic inhibitors would most likely not mimic the effects of HDAC9 gene deletion in obesity, since HDAC9 appears to regulate adipogenesis independent of its deacetylase enzymatic activity. Indeed, overexpression of a truncated C-terminal mutant of HDAC9 that lacks the enzymatic domain produced equivalent impairment of adipogenic differentiation as compared with the full-
length molecule, while overexpression of an N-terminal mutant that lacks the nuclear localization sequence but possesses full enzymatic activity had no effect. Consistent with this notion, a recent study reported that a class II HDAC enzymatic inhibitor, MC1568, did not improve metabolic disease in obese mice. Class II HDACs (HDAC 4–7, 9, 11) exhibit weak or no histone deacetylase enzymatic activity and are considered as pseudoenzymes; their deacetylase activity relies primarily on recruitment of catalytically potent class I HDACs (HDAC 1–3, 8, 10). Interestingly, deacetylase enzymatic activity likewise does not underlie all of the functional effects of class I HDACs. For example, HDAC3, a class I HDAC, regulates gene expression and metabolic activities in the liver, but its histone deacetylase activity is completely dispensable for these functions. Thus, it is perhaps not surprising that HDAC enzymatic inhibitors do not always phenocopy the effects of HDAC knockdown or gene deletion, and in some cases, may yield opposite results.

Evidence is emerging that HDAC9, like other class II and class I HDACs, modulates gene expression by interacting with nuclear repressor complexes. HDAC9 likely acts as a scaffold protein in the cell nucleus by physically interacting with transcriptional repressors and transcription factors (USF1, PPARγ, etc.) at discrete transcription sites to influence gene expression. HDAC9 also regulates epigenetic histone modifications at select gene targets to influence gene expression. More research is required to define HDAC9’s nuclear binding partners in order to understand how it impairs adipogenesis; this information can then be used to develop a therapeutic strategy that is likely to be effective. It is important to point out that there is a clear gene-dose effect of HDAC9 to influence metabolic disease in chronic high fat fed mice. HDAC9 heterozygote mice display an “intermediate phenotype” with regard to many aspects of diet-induced obesity; they gain less weight (not shown) and exhibit lower plasma leptin levels as compared with wild-type mice (Fig. 2A), and the extent of ectopic lipid accumulation in the liver is dramatically reduced in the heterozygotes, approaching that seen in HDAC9 homozygous knockout mice (Fig. 2B). This remarkable gene-dose effect of HDAC9 expression on metabolic parameters suggests that a moderate degree of inhibition of HDAC9, such as is commonly achieved with pharmacotherapy, could favorably impact obesity-related metabolic disease.

How HDAC9’s molecular actions are functionally linked to the global metabolic state in obesity remains to be ascertained. It should be pointed out that, in addition to adipose tissue, HDAC9 is expressed in other metabolically relevant tissues, including liver, skeletal muscle, and pancreas as well as in immune cells. This expression pattern suggests that HDAC9 is globally positioned to influence metabolic disease by influencing multiple target tissues and cells. Our laboratory is currently examining the tissue-specific role of HDAC9 in metabolic disease during chronic high fat diet-induced obesity and in genetic models of obesity. Hopefully, these investigations will reveal which tissues are critical in transducing the actions of HDAC9 in obesity, which could be important from a therapeutic perspective. In addition, HDAC9 is expressed in vascular cells and tissues. Elevated vascular HDAC9 expression was reported to be associated with aortic and femoral atherosclerosis as well as carotid artery disease and large vessel ischemic stroke in humans. Moreover, a recent genome-wide association study found two single nucleotide polymorphic (SNP) variants of HDAC9 to be strongly linked to large blood vessel disease in humans. These findings raise the intriguing possibility that therapeutically targeting HDAC9 in obesity could also favorably modulate the development of cardiovascular complications, a major cause of morbidity and mortality in this population.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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