Lsd1, a metabolic sensor of environment requirements that prevents adipose tissue from aging

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ABSTRACT
Understanding development and maintenance of beige adipocytes provide exciting insights in establishing novel therapies against obesity and obesity-associated disorders. Lysine-specific demethylase 1 (Lsd1) is an epigenetic eraser required for differentiation and function of adipocytes. Lsd1 is involved in early commitment of preadipocytes, but dispensable for terminal differentiation of white adipose tissue (WAT). In mature adipocytes, Lsd1 responds to different environmental stimuli to alter metabolic function and enable proper thermogenic and oxidative response. Exposure to cold leads to Lsd1 upregulation and subsequent beiging of WAT. Oppositely, Lsd1 levels decline during aging resulting in a conversion of beige into white adipocytes, associated with loss of thermogenic properties of WAT. Lsd1 maintains beige adipocytes by controlling the expression of the nuclear receptor peroxisome proliferator-activated receptor α. In summary, our studies not only provided insights into the mechanism of age-related beige-to-white adipocyte transition, but also established Lsd1 as a sensor that enables thermogenic response in WAT.

KEYWORDS
aging; beige adipocyte; cold; Lsd1; Ppara

Introduction
Adipose tissue is an important regulator of energy balance and nutritional homeostasis. White adipose tissue (WAT) is composed of white adipocytes that store excess triglycerides as large lipid droplets and hydrolyze them upon high energy demand. In addition to classical white adipocytes, WAT also contains thermogenically active beige adipocytes. These cells are characterized by abundance of mitochondria, multilocular lipid droplets, and specific expression of uncoupling protein 1 (Ucp1) that converts chemical energy into heat. Due to their capacity to counteract metabolic diseases including obesity and type 2 diabetes, beige adipocytes have recently been recognized as potential therapeutic targets. The number of beige adipocytes in murine WAT varies with anatomic location of the fat depots or with age. For instance, inguinal WAT (ingWAT) of young mice contains larger proportion of beige adipocytes than what is observed for epididymal WAT (epWAT). Beige adipocyte appearance can be induced by adrenergic stimulation or exposure to cold, whereas their number declines upon high-caloric diet or low physical activity. With age, Ucp1-positive beige adipocytes are progressively lost from WAT depots and converted into white fat cells. Despite the high interest in understanding beige adipocyte development and maintenance, the molecular mechanisms orchestrating emergence and age-related beige-to-white adipocyte transition remain unknown.

Lsd1, an early effector of preadipocyte commitment
Adipogenesis requires a sequential activation and repression of genes regulated by pro-adipogenic (e.g. Cebps,
Fabp4, Ppary) and anti-adipogenic (e.g., Gata2) factors. To investigate the involvement of Lsd1 in adipogenesis, we ablated Lsd1 at different time points during mouse development or in vitro differentiation. First, to induce Lsd1 ablation in adipocyte precursors we used the Fatty acid binding protein 4 (Fabp4)-Cre deleter strain. Fabp4 expression indeed marks a population of adipocyte progenitors in white and brown adipose tissue, but it is also expressed in other cell types including mature adipocytes and endothelial cells. We demonstrated that Lsd1 is essential for the development of both white and beige adipocytes, since Lsd1 knockout mice were completely devoid of visceral and subcutaneous fat. These data were corroborated by in vitro differentiation of primary preadipocytes isolated from the stromal vascular fraction of various fat depots. In these cells, Lsd1 ablation before adipogenic induction abolished formation of mature adipocytes and strongly downregulated the expression of pro-adipogenic transcription factors Cebpβ, Fabp4, Ppary, and Cebpα, as well as markers of mature adipocytes Adipoq, Agt, and Rtn. The control of early adipogenesis was dependent on Lsd1 demethylase activity, since treatment with Lsd1-specific inhibitor faithfully recapitulated the phenotype observed in knockout cells. Our findings are in full agreement with Musri et al. who showed that in 3T3-L1 cells Lsd1 demethylates H3K9me2 histone mark at the Cebpα promoter to activate gene transcription (Fig. 1).

Although Lsd1 plays a role in early adipogenesis, it is dispensable for maturation of adipocytes at later stages of differentiation. Indeed, our data showed that Lsd1 ablation or inhibition 3 d after induction of differentiation did not affect formation of adipocytes in tissue culture. Similar results were obtained by using Adipoq-Cre deleter strain to ablate Lsd1 in differentiating adipocytes. At this developmental stage, Lsd1 ablation did not affect WAT development. Together, our data demonstrated that the epigenetic modifier Lsd1 is required for early commitment of preadipocytes. Maturation of adipocytes is not dependent on Lsd1, most probably due to the fact that induced adipocyte-specific transcription factors are sufficient to accomplish terminal differentiation (Fig. 1).

Lsd1 governs the induction of beige adipocytes

Since we found that Lsd1 was involved in WAT development, we questioned whether it would also contribute to the induction of thermogenic beige adipocytes upon cold or β3-adrenergic stimulation. Interestingly, Lsd1 protein levels were increased in ing- and epWAT of wild-type mice after cold exposure or treatment with the β3-adrenergic agonist CL316,243, indicating that Lsd1 might mediate thermogenic response in WAT (Fig. 2). To further investigate these findings, we generated mice with adipocyte-specific Lsd1 ablation using inducible Cre recombinase under the control of Adipoq promoter (Adipoq-CreER). We found that in the absence of Lsd1, ingWAT failed to induce beige adipocytes upon cold stimulation, supporting our hypothesis that Lsd1 mediates thermogenic response in mice.

Opposite from what was observed upon Lsd1 ablation, in transgenic Lsd1 overexpressing mice, elevated Lsd1 levels were sufficient to mimic thermogenic response in the absence of cold stimulus. Indeed, these mice displayed a range of morphologic and metabolic phenotypes in WAT associated with an increased number of multilocular mitochondria-rich beige adipocytes expressing high levels of thermogenic (Prdm16, Pgc-1α, Ucp1, Ucp2, and Ucp3) and oxidative markers (Nrf1, Cpt1b, and Cox8b). Due to increased thermogenic and uncoupling capacities, transgenic mice exhibited higher body temperature compared with their control littermates, which limited their weight gain and adiposity of WAT depots upon high-fat diet. In addition, these mice were characterized by decreased blood glucose levels, improved glucose tolerance, and increased insulin sensitivity, indicating decreased propensity to develop type-2 diabetes. In conclusion, elevated Lsd1 levels in mice mimic the effects of cold exposure or β3-adrenergic

Figure 1. Lsd1 promotes early adipogenesis in WAT but is dispensable for terminal differentiation. During early adipogenesis and commitment of preadipocytes, Lsd1 enzymatic activity plays an essential role in activating the expression of key adipogenic genes through H3K9me2 demethylation at their promoters. However, Lsd1 is not required during maturation of committed adipocytes.
treatment to promote thermogenic and oxidative capacities of WAT.

Using genome-wide transcriptome analysis, we showed that Lsd1-mediated metabolic adaptation to cold is governed by increased expression of Tfam, regulator of mitochondrial biogenesis, as well as genes encoding the mitochondrial respiratory chain subunits. Lsd1 directly regulates the expression of these genes in cooperation with Nrf1, a key factor controlling mitochondrial biogenesis. Together, these experiments show that Lsd1 act as a master regulator of cold stimulation in WAT by controlling a broad thermogenic gene network (Fig. 3).

**Lsd1 prevents loss of beige adipocytes**

Since we observed increased Lsd1 protein expression together with beige fat formation in response to cold or β3-adrenergic stimulation, we hypothesized that Lsd1 levels might also be modulated upon decline of beige adipocytes in WAT. Indeed, we observed that whitening of adipogenic depots with age was accompanied by decreased Lsd1 expression in both ep- and ingWAT of aged wild-type mice (Fig. 2). We therefore investigated the role of Lsd1 in the regulation of age-related beige-to-white adipocyte transition by using adipocyte specific gain- and loss of function mouse models. We found that Lsd1 overexpression in adipocytes was sufficient to maintain the pool of beige fat cells with age, whereas Lsd1 ablation led to a premature loss of beige adipocytes, with a complete conversion into white adipocyte appearance at only 10 weeks of age. Loss of beige adipocytes was not caused by apoptosis, but by beige-to-white transition, as confirmed by tracing the fate of Ucp1-expressing beige adipocytes upon Lsd1 ablation. For this purpose, we generated Lsd1 knockout mice expressing green fluorescent protein (GFP)-diphtheria toxin receptor (Dtr) fusion construct under the control of the Ucp1 promoter (Ucp1-Dtr-GFP reporter strain). In such mice, GFP-Dtr protein was selectively addressed to the

**Figure 2.** Lsd1 acts as a sensor that integrates different environmental stimuli to enable proper thermogenic and oxidative responses of beige adipocytes. In young mice, physiologic levels of Lsd1 maintain thermogenic properties in WAT. However, with age, Lsd1 levels progressively decline concomitant with beige-to-white adipocytes transition. In WAT, Lsd1 levels can be modulated by different environmental inputs. Cold induces Lsd1 expression together with thermogenic response in WAT, whereas food excess leads to decreased Lsd1 protein levels and to accelerated beige-to-white adipocytes transition.

**Figure 3.** Lsd1 controls beige-selective transcription Pparα and a broad network of metabolic genes to regulate thermogenic properties of WAT. Under physiologic conditions, Lsd1 activates Pparα expression via H3K9me2 demethylation. In addition, in complex with Nrf1, Lsd1 regulates oxidative capacities of WAT. Upon genetic ablation or declined Lsd1 levels caused by age, Pparα expression is disabled, leading to beige-to-white adipocyte transition. On the other hand, increased Lsd1 levels in transgenic mice or via cold or β3-adrenergic stimulation leads to increased expression of OXPHOS and thermogenic genes, and activates beiging of WAT.
membrane of Ucp1-positive cells, allowing us to trace beige fat cells even after they adopted white adipocyte morphology. Since unilocular adipocytes in Lsd1-ablated ingWAT expressed GFP, we concluded that Ucp1-expressing beige adipocytes were formed, but rapidly acquired white adipocyte appearance. These findings demonstrated that Lsd1 is required for maintenance of beige adipocytes in WAT.\textsuperscript{21} Using a similar approach, we showed that Lsd1 is not only responsible for the maintenance of endogenous / basal beige adipocytes, but also for conserving the pool of cold-induced beige adipocytes. Ablation of Lsd1 after 10 d of cold-treatment rendered the mice incapable of maintaining beige adipocytes, and their ingWAT displayed a white adipocyte morphology devoid of thermogenic abilities even in conditions of continued cold stimulus.\textsuperscript{21} Next, we questioned the molecular mechanism through which Lsd1 allows the maintenance of beige adipocytes. Our transcriptome analysis in Lsd1-ablated ingWAT revealed the importance of the Peroxisome proliferator-activated receptor (Ppar) signaling pathway in Lsd1-mediated maintenance of beige adipocytes in WAT.\textsuperscript{21} Ppars are ligand-regulated nuclear receptors that play important roles in fat differentiation and metabolism.\textsuperscript{27,28} In particular, Ppar\(\alpha\) seems to be more brown-specific, since it is expressed at higher levels in brown relative to white adipocytes.\textsuperscript{29,30} Since beige adipocytes share common metabolic features with brown adipocytes, we hypothesized that Ppar\(\alpha\) might contribute to the maintenance of beige adipocytes. Indeed, Ppar\(\alpha\) transcript levels were decreased upon loss of Lsd1 in ingWAT, indicating that Lsd1 is regulating Ppar\(\alpha\) expression. A detailed analysis of the Ppar\(\alpha\) locus by chromatin immunoprecipitation revealed that Lsd1 directly activates Ppar\(\alpha\) expression since Lsd1 was bound to the Ppar\(\alpha\) promoter together with p300 and PolII, leading to decrease in H3K9me2 levels (Fig. 3). In accordance, Ppar\(\alpha\) levels in WAT strongly decreased with age, similar to Lsd1. These observations are in accordance with literature data showing that Ppar\(\alpha\)-null mice display reduced number of beige adipocytes.\textsuperscript{31} Using pharmacological Ppar\(\alpha\) agonist\textsuperscript{32} and antagonist,\textsuperscript{33,34} we confirmed that Ppar\(\alpha\) mediates the effects of Lsd1 in maintaining beige adipocytes. Treatment of young Lsd1 knockout mice with Ppar\(\alpha\) agonist was sufficient to alleviate the premature beige-to-white adipocyte transition and to restore the transcript levels of beige fat markers. Oppositely, pharmacological inhibition of Ppar\(\alpha\) action in aged Lsd1 overexpressing mice abrogated maintenance of beige adipocytes.\textsuperscript{35} These findings are of particular interest, since they indicate that pharmacological modulation of Ppar\(\alpha\) signaling can be used as a therapeutic strategy to maintain beige adipose tissue during aging and preserve lean phenotype in mice.

**Conclusion**

Our work underlines important roles of Lsd1 in different aspects of WAT development, maintenance, and function. During early development Lsd1 is essential for the formation of adipose tissue (Fig. 1). In mature adipocytes, Lsd1 acts as a sensor that integrates different environmental stimuli to alter metabolic function and enable proper thermogenic and oxidative responses (Fig. 2). Exposure to cold leads to Lsd1 upregulation and subsequent beiging of WAT, whereas during aging Lsd1 levels decline resulting in a loss of thermogenic properties of WAT (Fig. 3). Our study has for the first time implicated an epigenetic eraser Lsd1 in white adipose tissue metabolism. Further experiments are required to identify how Lsd1 expression levels are regulated by environmental cues and what kind of factors convey information from cell surface to the nucleus. Unraveling mechanisms governing development and maintenance of beige adipocytes provide exciting insights in establishing novel therapies against obesity and obesity-associated disorders.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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