Impaired histone deacetylases 5 and 6 expression mimics the effects of obesity and hypoxia on adipocyte function

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ABSTRACT

Objective: The goal of the study was to investigate the role of histone deacetylases (HDACs) in adipocyte function associated with obesity and hypoxia.

Methods: Total proteins and RNA were prepared from human visceral adipose tissues (VAT) of human obese and normal weight subjects and from white adipose tissue (WAT) of C57Bl6-Rj mice fed a normal or high fat diet (HFD) for 16 weeks. HDAC activity was measured by colorimetric assay whereas the gene and protein expression were monitored by real-time PCR and by western blotting, respectively. RNA interference (RNAi) from white adipose tissue (WAT) of C57Bl6-Rj mice fed a normal or high fat diet (HFD) for 16 weeks. HDAC activity was measured by colorimetric assay whereas the gene and protein expression were monitored by real-time PCR and by western blotting, respectively. RNA interference (RNAi) was used to silence the expression of genes in 3T3-L1 adipocytes.

Results: Total HDAC activity was decreased in VAT and WAT from obese individuals and from mice fed a HFD, respectively. The HDAC activity reduction was associated with decreased HDAC5/Hdac5 and HDAC6/Hdac6 expression in human and mice adipocyte fraction. Similarly, hypoxia hampered total Hdad activity and reduced the expression of Hdad5 and Hdad6 in 3T3-L1 adipocytes. The decrease of both Hdad5 and Hdad6 by hypoxia was associated with altered expression of adipokines and of the inducible cAMP early repressor (Icer), a key repressor that is defective in human and mice obesity. Silencing of Icer in adipocytes reproduced the changes in adipokine levels under hypoxia and obesity, suggesting a causative effect. Finally, modeling the defect of the two Hdacs in adipocytes by RNAi or selective inhibitors mimicked the effects of hypoxia on the expression of Icer, leading to impairment of insulin-induced glucose uptake.

Conclusion: Hdad5 and Hdad6 expression are required for the adequate expression of Icer and adipocyte function. Altered adipose expression of the two Hdacs in obesity by hypoxia may contribute to the development of metabolic abnormalities.

Keywords Histone deacetylases; Adipocytes; Adipokines; Obesity; Insulin resistance

1. INTRODUCTION

Insulin resistance is a key feature of obesity and is involved in the development of type 2 diabetes, fatty liver disease, cardiovascular disease, and cancer [1–4]. Insulin resistance is associated with altered production of several adipokines (i.e. bioactive secreted products from adipocytes) that regulate insulin sensitivity and energy metabolism [5–7]. These adipokines include interleukin 6 (IL6), nicotinamide phosphoribosyltransferase (NAMPT, also called visfatin), leptin (LEP), angiotensinogen (AGT), Lipocalin 2 (LCN2), adiponectin (ADIPOQ), resistin (RETN), and SERPINE1 (also called plasminogen activator inhibitor type 1) [5,6,8]. These adipokines have been shown to induce insulin resistance in rodents [5,9,10]. In both human and mice obesity, hypoxia is thought to contribute to impaired adipokine production [11–14]. Indeed, visceral adipose tissue (VAT) from obese subjects is characterized by impaired blood flow, defective capillary density, and impaired O2 partial pressure [12,14]. Exposing mouse adipocytes to hypoxia leads to reduced expression of

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Figure 1: Measurement of Hdac/HDAC activity and expression from adipose tissues, adipocytes and svf in diet-induced obese mice, non-obese and obese individuals. A) Hdac activity in WAT of mice fed with regular or HFD. Total proteins were prepared from WAT of mice that were fed with regular (open bars, Chow, n = 10 mice) or HFD (filled bars, HFD, n = 10 mice). HDACs activity was measured by direct colorimetric assay kit (Epigentek). Data are the mean ± SEM of 3 independent experiments (*p < 0.05). Quantiﬁcation of B) Class I Hdac1/2/3/8, C) Class IIa Hdac4/5/7/9, D) Class IIb Hdac6/10, and E) Class IV Hdac11 mRNA levels. The mRNA of F) Hdac5, G) Hdac6, and H) Hdac9 was quantified by PCR in adipocytes and stroma vascular fraction (SVF) that were collected from WAT from control (open bars, Chow) and obese mice (filled bars, HFD). The Hdac mRNA levels were determined by quantitative real-time PCR and were normalized against the housekeeping acidic ribosomal phosphoprotein P0 gene (Rplp0). Similar results were obtained while normalizing against TBP. The results were expressed as the fold changes over the controls. Data are the mean of ±SEM of 3 independent experiments (***P < 0.001; **P < 0.01; *P < 0.05). H) HDAC activity in VAT of non-obese and obese individuals. Total protein concentrations were prepared from VAT of non-obese (open bars, NO) or obese individuals (filled bars, Obese) and were subjected to colorimetric assay kit (Epigentek). Data are the mean ± SEM of 3 independent experiments (**P < 0.01). Quantification of I) Hdac5, J) Hdac6, and K) Hdac9 mRNA in adipocytes and SVF from VAT of non-obese (open bars, NO) or obese individuals (filled bars, Obese). The HDAC mRNA levels were determined by quantitative real-time PCR. The mRNA levels were normalized against the RPLP0 and were expressed as the fold changes over the controls. Data are the mean of ±SEM of 3 independent experiments (**P < 0.01; *P < 0.05).
Adipoq, Agt, Lep, Nampt, and Retn, and, in contrast, to increased expression of Serpin e1, Ile, and Lcn2 [8,11]. However, the molecular mechanisms causing impaired adipokine production associated with hypoxia are still elusive. We postulate that both cAMP response element (CRE) binding protein activity in (CREB) and histone deacetylases are involved in these mechanisms. In support of this working hypothesis, hypoxia can stimulate CREB activity as observed in PC12 and lung cells [15,16]. Adipocyte CREB activity is increased in obesity, leading to increased abundance of the activating transcription factor 3 (ATF3) [17,18]. This increased ATF3 activity hampers the expression of Adipoq and glucose transporter GLUT4, ultimately leading to impairment in insulin-induced glucose uptake [17,18]. The CREB-dependent activation mechanism is initiated by reduction in the content of inducible cAMP early repressor (ICER), a natural antagonist of CREB and other cAMP-dependent transcription factors [17]. Reduction of ICER was found in adipocytes of human obese individuals and mice fed a high-fat diet (HFD) for 16 weeks [17]. In this study, therefore, we hypothesized that defective deacetylase activity may account for the collapse in the ICER level in obesity. Indeed, the expression of ICER is reported to be positively regulated by histone deacetylase activity (HDACs) in PC12 cells [19]. Overall, HDACs are pivotal in epigenetic mechanisms that permit gene expression adaptation to environmental changes [20]. There are 3 classes of HDACs [21,22]: classes I, II and IV. Class I HDACs comprises HDAC1, HDAC2, HDAC3, and HDAC8. Class II HDACs is divided into subclass Ila (HDAC4, HDAC5, HDAC7, and HDAC9) and subclass Iib (HDAC6 and HDAC10). Class IV contains HDAC11 only. So far, selective inhibition of HDACs is a strategy for treating many cancers [22]. Additionally, there is emerging evidence implicating HDac activity in the control of energy metabolism, thus opening an avenue for future targets in metabolic diseases [23]. In the hypothalamus of obese mice fed a HFD, the expression of Hdacs, including Hdad5, is modified when compared to that of mice fed a Chow diet [24,25]. Hdad5 is required for hypothalamic leptin signaling and food intake, as Hdad5 knockout mice display defective hypothalamic leptin signaling and are more prone to diet-induced obesity compared to wild-type mice [25]. Given the role of HDacs in obesity, we hypothesized the contribution of HDacs to the changes in adipokine expression elicited by hypoxia and obesity-associated adipocyte dysfunction.

2. MATERIAL AND METHODS

2.1. Materials
Trichostatin (TSA), tubastatin and LMK293 were obtained from Sigma-Aldrich (St. Louis, MO).

2.2. Biopsies and RNA preparation
Total RNA was extracted from epididymal white adipose tissue (WAT) of mice fed a HFD (n = 10) or normal Chow diet (n = 10). WAT isolation was performed in euthanized animals in accordance to the Swiss legislation for animal experimentation. Approximately 5 cm² of WAT was obtained at the level of the omentum from five obese Caucasian women (BMI > 35 kg/m²) who were referred for weight reduction surgery and five non-obese Caucasian women (24 < BMI < 28 kg/m²) [17]. All patients provided informed consent, and the study was approved by the institutional review board [17]. The criteria for exclusion and phenotyping are those previously described [17]. Total RNA was isolated from adipose tissues and different cell fractions with the TriPure isolation reagent (Roche) as previously described [17]. Procedure for preparation of adipocytes and SVF fractions was done as described [17].

2.3. Cell culture and transfection
Culture and differentiation of 3T3-L1 cells were conducted as described [17]. Briefly, 3T3-L1 cells were grown and maintained in Dulbecco’s modified Eagle’s medium high glucose containing 50 units/ml penicillin, 50 μg/ml streptomycin, and 10% fetal calf serum (FCS) in a 10% CO₂ environment. At postconfluence (2 days), the cells were differentiated by adding to the culture medium, isobutylmethylxanthine (500 μM), dexamethasone (25 μM), and insulin (4 μg/ml) for 3 days and then treated with insulin for 3 more days. The medium was then changed every 3 days until the cells were fully differentiated, typically by 10 days. The 19-nt small interfering RNA (siRNA) duplex against Icer (slicer) is described elsewhere [17]. The siRNA duplexes, targeting the GFP (5’-CGCTGACCCTGAAGTTCAT-3’), the mouse Hdad5 (5’-GCAA-GATTCTACAAGGAT-3’), and Hdad6 (5’-CCAGGAGCTTCCAAGAT-3’) were purchased from Microsynth (Balgach, Switzerland). For silencing Icer, Hdad5, and Hdad6, on day 7 post-differentiation, 3T3-L1 adipocytes were electroporated with siRNAs using the GenePulser XCell (Bio-Rad) as previously described [17].

2.4. Western blotting and quantitative PCR
Protein extracts, western blotting, and real-time quantitative PCR were conducted as previously described [17]. PCR assays were carried out on a BioRad MyiQ Single-Color Real-Time PCR Detection System using the BioRad iQ SYBR Green Supermix, with 100 nM primers and 1 μl of template per 20 μl of PCR and an annealing temperature of 60°C. The primer sequences are available in the supplementary materials.

2.5. 2-Deoxyglucose (2-DG) uptake assay
2-deoxy-d-[1,2-3H]glucose (2-[^3H]DG, 26.2 Ci/mmol) uptake assays were conducted on fully differentiated 3T3-L1 adipocytes (days 7 and 8) as previously described [17]. Adipocytes were treated without (basal) or with insulin 10 nM for 10 min. 2-[^3H]DG (0.1 μCi; final concentration, 0.01 mmol/l) and 5 mM cold 2-DG were then added for an additional 10 min at 37°C. 2-DG uptake was terminated by washing the cells three times with ice-cold PBS containing 10 mM glucose. Subsequently, cells were lysed in 1% (wt/vol) SDS and 0.2 M NaOH. Incorporation of radioactivity was measured by liquid scintillation spectrometry.

2.6. Statistical analysis
The experiments including two groups were analyzed by t-test or with the non-parametric equivalent Wilcoxon.
Brief Communication

3. RESULTS

3.1. HDAC5/Hdac5 and HDAC6/Hdac6 mRNA levels are reduced in adipocytes from obese human subjects and from obese mice

The total Hdc activity was monitored in WAT of mice that were fed a chow diet or a HFD for 16 weeks. We and others have shown previously that mice gain more weight, develop more adipose tissue, and develop systemic insulin resistance when fed a HFD [17]. The total Hdc activity was significantly decreased in the WAT of HFD mice (Figure 1A). Decreased Hdc activity was associated with a significant drop of classes IIa and IIb, Hdc5 and Hdc6 expression, respectively (Figure 1C,D). The level of all Hdc mRNA as well as the reduction of Hdc5 and Hdc6 expression was confirmed while the qRT-PCR analyses were normalized against the TATA box binding protein mRNA, for which we found that the level was also stable among the mice. In contrast, Hdc9 expression was significantly increased whereas the expression of class I Hdcas (Hdc1-3 and Hdc8) was unchanged in obese vs control mice (Figure 1B). The diminution of the two Hdcas had originated in the mice adipocyte fraction (Figure 1E,F), whereas the increased expression of Hdc9 was observed only in the adipose stromal-vascular fraction (SVF) of obese mice (Figure 1G). Similar results were found in human adipocytes and in SVF fractions of VAT from obese individuals (Figure 1H–K). The collapse of total Hdc activity (Figure 1H) was associated with a decrease in HDAC5 and HDAC6 expression in human adipocyte fraction (Figure 1J). As observed in the mice, the Hdc9 expression was augmented in SVF (Figure 1K). These results indicate a decrease in adipocyte expression of HDAC5 and HDAC6 in human and mouse obesity.

3.2. Hypoxia mimics the reduction of Hdc5/HDAC5 and Hdc6/ HDAC6 mRNA and the loss of Icer activity in obesity

We hypothesized that the reduction of Hdc activity and mRNA level of Hdc5 and Hdc6 in adipocytes is produced by hypoxia. Hypoxia results in increased lactate release, which is associated with an increase of mRNAs of monocarboxylate transporters 1 and 4 (Mct1 and Mct4, respectively) [26]. Here, we confirmed that Mct1 and Mct4 levels were increased in 3T3-L1 adipocytes cultured under hypoxia for 24 h (Figure S1). Consistent with the induction of lactate metabolism by hypoxia, the expression of both lactate dehydrogenase gene (LdhA) and pyruvate dehydrogenase kinase 1 gene (Pdk1) were augmented (Figure S1). These hypoxia-induced changes were associated with decreased expression of both Hdc5 and Hdc6 (Figure 2A). Consistently, the reduction of both Hdc5 and Hdc6 in response to hypoxia was associated with a diminution of the total Hdc activity (Figure 2B). The expression of hypoxia-sensitive genes has been shown to rely on the increase of CREB activity [27,28]. Alteration of Icer expression may account for defective adipokine gene expression and increased CREB activity caused by hypoxia. The expression of Icer, therefore, was quantified in 3T3-L1 cells exposed to hypoxia. We found that hypoxia alleviated the expression of Icer mRNA under non-stimulatory conditions and in response to IBMX and Forskolin (Figure 2C). Parallel to the diminution of Icer expression, as expected, the CREB target Akt3 level was increased (Figure 2D), confirming an increase of CREB transcriptional activity by hypoxia. This result further suggested that reduced Icer expression modifies the expression of hypoxia-inducible adipokines. Indeed, hypoxia affects the expression of numerous genes encoding key adipokines involved in insulin resistance, including Il6, Nampt, Apg, Lep, Adipq, Lcn2, Retn, and Srp1. Culture of 3T3-L1 adipocytes under hypoxia significantly increased the level of Il6, Srp1, and Lcn2, whereas it reduced the mRNA of Retn, Apg, Lep, Nampt, and Adipq (Figure 2E). The expression of these adipokines was monitored in 3T3-L1 adipocytes in which the expression of Icer was silenced by small interfering RNAs (siCer). The efficiency and specificity of siCer for reducing Icer abundance in 3T3-L1 adipocytes has been reported in our previous study [17]. Here, we showed that Icer silencing significantly increased the expression of Il6, Lcn2, and Srp1, whereas that of Retn, Apg, Lep, Nampt, and Adipq was significantly reduced (Figure 2F). In humans, similar changes in adipokine levels were observed in the VAT of obese subjects (Figure S2), in whom we previously found a reduction in Icer activity [17]. Altogether, these data support a role for adipocyte Hdc5 and Hdc6 in the control of Icer level and thereby in hypoxia-sensitive adipokine expression in obesity.

3.3. Hdc5 and Hdc6 levels are required for the expression of Icer and glucose uptake

We next investigated the role of Hdc5 and Hdc6 levels on Icer expression. The efficient silencing of both Hdc5 and Hdc6 in 3T3-L1 adipocytes using small interfering RNAs duplexes (siH5 and siH6) was shown by Western Blotting analyses (Figure 3A). The reduction of Hdc5 or Hdc6 level diminished the expression of Icer in 3T3-L1 adipocytes (Figure 3B). The induction of Icer by cAMP raising agents IBMX and Forskolin was also attenuated upon silencing of both Hdc5 (Figure S3a). This result was confirmed using Hdc inhibitors. The pan-HDAC inhibitor trichostatin (TSA) attenuated basal Icer expression (Figure S3b) and the induced expression of repressor in response to cAMP raising agents IBMX and Forskolin in 3T3-L1 adipocytes (Figure S3c). LMK 235 and tubastatin A are highly selective Hdc5 and Hdc6 inhibitors, respectively [33,34]. Culture of 3T3-L1 adipocytes with either LMK 235 or tubastatin A reproduced the effects of TSA on the expression of Icer (Figure S3c). In agreement with the decrease of Icer level by siH5 or siH6, we observed that the expression of Akt3 and Glut4 was increased (Figure 3C,D). As anticipated, we found that the reduction of Icer by siH5 or siH6 was accompanied by an impairment of insulin-induced DOG uptake in 3T3-L1 adipocytes (Figure 3E), thus supporting a role for HDACS and HDAC6 in the glucose transport in a mechanism involving ICER (Figure 4).

4. DISCUSSION

Adipose reduction of Icer may be instrumental in the link between obesity and systemic insulin resistance. In the present study, we showed that the reduction of Icer in adipocytes of both obese mice and obese human subjects is the consequence of impaired HDAC5/Hdac5 and HDAC6/Hdac6 expression. Indeed, among the 11 Hdcas expressed in adipose tissue, only these two enzymes were down regulated, which was sufficient to lead to an overall reduction of Hdc activity and a reduction of Icer expression. This result was further supported by the silencing of Hdc5 or Hdc6 in 3T3-L1 adipocytes that also hampered Icer expression. Post-translational modifications can modulate nuclear activity and translocation of transcriptional regulators [35,36]. We speculate that the defective activity of the two Hdcas in adipocytes leads to re-acetylation and thereby inhibition of some transcription factor(s) required for the Icer expression. In this case, increased Icer expression may result from silencing the transcriptional repressor. This mechanism has been suggested for the positive regulation of Icer by HDAC activity in PC12 cells [19]. We speculate that HDAC5 and HDAC6 activate the expression of Icer in adipocytes via a similar mechanism. Such regulation by the two HDACs may further rely on a concerted mechanism. The two HDACs may be co-localized in the nucleus within the gene coding for Icer. HDAC5 and HDAC6 can translocate from the nucleus to the cytosol depending on the cell type and stress condition
Hdac5, Hdac6, and proteins were subjected to Western blotting experiments for the quantification directed against GFP (siCtl) or with siRNAs against Hdac5 (siH5) or Hdac6 (siH6). Total pocytes were electroporated with 5 nmol of control small interfering RNA duplexes Ef Icer represents a passive transcriptional repressor that prevents binding of the CREB transcriptional activator within CRE, leading to gene silencing. Therefore, it is possible. This was shown for HDCA6 and HDAC9 in GnRH neuronal cells for modulating cell movement and survival [40]. Moreover, interaction of class Ila and class Iib HDACs is possible. This was shown for HDCA6 and HDAC9 in GnRH neuronal cells for modulating cell movement and survival [40]. Therefore, it is possible that HDAC5 and HDAC6 act in concert for positively regulating the expression of Icer via an indirect mechanism involving similar nuclear or cytoplasmic targets in adipocyte. In contrast to adipocytes, in SVF of obese subjects and mice, ICER/Icer expression is increased [17]. We show in this study that increased ICER/Icer is associated with the elevation of Hdad5/Hdad6 expression. Future studies are needed to determine whether HDAC9 accounts for the increase of Icer and thereby impacts cell function in SVF.

Icer is a passive transcriptional repressor that prevents binding of the CREB transcriptional activator within CRE, leading to gene silencing.

The antagonistic effect of ICER is a major mechanism that permits cells to return to their basal state upon cAMP-raising conditions such as fasting and glucagon and beta-adrenergic stimulation. In obesity, the decrease of ICER/Icer in adipocytes increases the binding of CREB within the CRE of its target genes [17,18], increasing the transcriptional activity of CREB increases and the expression of its target ATF3 [17,18]. Herein, we showed not only ATF3 but also ILE, Serpine1, and Lcn2 are increased, providing further evidence for a defective CREB pathway in obesity. In contrast, the expression of Retn, Agt, Lep, Nampt, and Adipoq were decreased upon Icer silencing. This suggests that they are indirect targets of CREB.

The dysregulation of adipokines and altered Hdac5 and Hdac6 levels in obesity are mimicked by hypoxia. We showed in 3T3-L1 adipocytes that the changes in adipokine levels caused by hypoxia are associated with impaired expression of the two Hdacs. We also found that adipokine dysregulation caused by hypoxia is the consequence of defective ICER activity. Silencing Icer in 3T3-L1 adipocytes mimicked the effect of hypoxia on the expression of adipokines. Finally, we propose that the reduction in Icer expression couples defective Hdac5 and Hdac6 activities to perturbed expression of adipokines and adipocyte dysfunction caused by hypoxia and obesity (Figure 4). Global methylation of histones and DNA is modified in adipose tissue from obese subjects [41,42]. The main function of HDAC is to deacetylate histones and non-histone proteins. Moreover, each HDAC can have several targets. A reduction in Hdac5 and Hdac6 expression, therefore, may lead to re-acetylation of several cytosolic and/or nuclear proteins. In other words, the acetylyme of adipocyte may be modified in obesity. Some nuclear re-acetylated proteins may contribute directly or indirectly to the deregulation of Icer and thereby to impaired adipokine production, adipocyte dysfunction, and, ultimately, systemic insulin resistance in the context of obesity.

Our study supports the requirement of maintaining Hdac5 and Hdac6 levels for optimal adipocyte function including glucose uptake and adipokine genes expression. Appropriate expression of Hdac5 has been shown to be required for energy metabolism in mice [25]. The expression of Hdac5 is indeed decreased in the hypothalami of obese mice [25]. Genetic and pharmacological inhibition of hypothalamic Hdac5 impairs leptin signaling and has deleterious effects on food intake and body-weight control [25]. Inhibition of some Hdacs, therefore, may be detrimental for energy metabolism in human. While inhibition of the class I HDAC Hdac3 has been seen as a promising therapeutic strategy for diabetes [23,43–46], inhibition of Hdac8, in contrast, may cause insulin resistance [47]. Moreover, there is a large spectra of class II

![Figure 3: Effect of silencing of Hdac5 and Hdac6 levels in 3T3-L1 adipocytes.](image)

A) Efficiency of siRNAs on Hdac5 and Hdac6 content by Western Blotting. 3T3-L1 adipocyte dysfunction caused by hypoxia is the consequence of defective ICER activity. Silencing Icer in 3T3-L1 adipocytes mimicked the effect of hypoxia on the expression of adipokines. Finally, we propose that the reduction in Icer expression couples defective Hdac5 and Hdac6 activities to perturbed expression of adipokines and adipocyte dysfunction caused by hypoxia and obesity (Figure 4). Global methylation of histones and DNA is modified in adipose tissue from obese subjects [41,42]. The main function of HDAC is to deacetylate histones and non-histone proteins. Moreover, each HDAC can have several targets. A reduction in Hdac5 and Hdac6 expression, therefore, may lead to re-acetylation of several cytosolic and/or nuclear proteins. In other words, the acetylyme of adipocyte may be modified in obesity. Some nuclear re-acetylated proteins may contribute directly or indirectly to the deregulation of Icer and thereby to impaired adipokine production, adipocyte dysfunction, and, ultimately, systemic insulin resistance in the context of obesity.

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HDAC inhibitors that are currently used for the treatment of cancers [22]. For example, belinostat, abexinostat, and trichostatin target several HDACs including HDAC5 and HDAC6 [22,34]. Therefore, it is possible that inhibitors targeting Hda5 and Hda6 affect adipose function and ultimately lead to insulin resistance. For optimal therapeutic metabolic impact, our study suggests the need of refining highly specific HDAC inhibitors with low affinity for HDAC5 and HDAC6.

5. CONCLUSION

We have identified altered adipocyte Hda5 and Hda6 expression in obesity and associated hypoxia. Modeling the defect of the two Hda5 in adipocytes by genetic silencing and selective inhibitors mimicked the effect of hypoxia and obesity on the expression of key adipokines and of Icer, the key regulator of adipokine transcription. We believe that our findings provide a significant hint for better understanding the mechanism responsible of adipose dysfunction, obesity related insulin resistance, and metabolic complications.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.molmet.2016.09.011.

REFERENCES

[1] Reaven, G.M., 1988. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 37(12):1595-1607.
[2] Moller, D.E., Flier, J.S., 1991. Insulin resistance—mechanisms, syndromes, and implications. New England Journal of Medicine 325(13):938—948. http://dx.doi.org/10.1056/NEJM199109263251307.
[3] Kahn, S.E., Hull, R.L., Utzschneider, K.M., 2006. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 444(7121):840—846. http://dx.doi.org/10.1038/nature05482.
[4] Reaven, G.M., 1995. Pathophysiology of insulin resistance in human disease. Physiological Reviews 75(3):473-486.
[5] Guerre-Millo, M., 2004. Adipose tissue and adipokines: for better or worse. Diabetes & Metabolism 30(1):13-19.
[6] Ouchi, N., Parker, J.L., Lugus, J.J., Walsh, K., 2011. Adipokines in inflammation and metabolic disease. Nature Reviews Immunology 11(2):85—97. http://dx.doi.org/10.1038/nri2921.
[7] Guillemre, A., Verbasius, J.V., Puri, V., Czech, M.P., 2008. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nature Reviews Molecular Cell Biology 9(5):367—377. http://dx.doi.org/10.1038/nrm2391.
[8] Wang, B., Wood, I.S., Trayhurn, P., 2007. Dysregulation of the expression and secretion of inflammation-related adipokines by hypoxia in human adipocytes. Pflugers Arch 455(3):479—492. http://dx.doi.org/10.1007/s00424-007-0301-8.
[9] Law, I.K.M., Xu, A., Lam, K.S.L., Berger, T., Mak, T.W., Vanhoutte, P.M., et al., 2010. Lipocalin-2 deficiency attenuates insulin resistance associated with aging and obesity. Diabetes 59(4):872—882. http://dx.doi.org/10.2337/db09-2541.
[10] Sun, Q., Li, L., Li, R., Yang, M., Liu, H., Nowicki, M.J., et al., 2009. Over-expression of visfatin/PBEF/Nampt alters whole-body insulin sensitivity and lipid profile in rats. Annals of Medicine 41(4):311—320. http://dx.doi.org/10.1080/07853890902729760.
[11] Hosogai, N., Fukushima, A., Oshima, K., Miyata, Y., Tanaka, S., Segawa, K., et al., 2007. Adipose tissue hypoxia in obesity and its impact on adipocytekine dys-regulation. Diabetes 56(4):901—911. http://dx.doi.org/10.2337/db06-0911.
[12] Wood, I.S., de Heredia, F.P., Wang, B., Trayhurn, P., 2009. Cellular hypoxia and adipose tissue dysfunction in obesity. Proceedings of the Nutrition Society 68(4):371—377. http://dx.doi.org/10.1017/S0029665109009206.
[13] Trayhurn, P., 2013. Hypoxia and adipose tissue function and dysfunction in obesity. Physiological Reviews 93(1):1—21. http://dx.doi.org/10.1152/physrev.00017.2012.
[14] Ye, J., Gao, Z., Yin, J., He, Q., 2007. Hypoxia is a potential risk factor for chronic inflammation and adiponectin reduction in adipose tissue of ob/ob and dietary obese mice. American Journal of Physiology. Endocrinology and Metabolism 293(4):E1118—E1128. http://dx.doi.org/10.1152/ajpendo.00435.2007.
[15] r.d. Hypoxia selectively activates the CREB family of transcription factors in the in vivo lung. http://www.ncbi.nlm.nih.gov.gate2.inist.fr/pmc/articles/PMC2643223/. [Accessed 11 May 2016].
[16] Chang, J.H., Vuppulanchi, D., van Niekerk, E., Trepel, J.B., Schanen, N.C., Twiss, J.L., 2006. PC12 cells regulate inducible cyclic AMP (cAMP) element repressor expression to differentially control cAMP response element-dependent transcription in response to nerve growth factor and cAMP. Journal of Neurochemistry 99(6):1517—1530. http://dx.doi.org/10.1111/j.1471-4159.2006.04196.x.
[17] Favre, D., Le Gouill, E., Fahmi, D., Verdumo, C., Chinetti-Gbaguidi, G., Staels, B., et al., 2014. Impaired expression of the inducible cAMP early repressor accounts for sustained adipose CREB activity in obesity. Diabetes 63(12):3169—3174. http://dx.doi.org/10.2337/db14-0170.
[18] Qi, L., Saberi, M., Zmuda, E., Wang, Y., Altarejos, J., Zhang, X., et al., 2009. Adipocytokines promote insulin resistance in obesity. Cell Metabolism 9(3):277—286. http://dx.doi.org/10.1016/j.cmet.2009.01.006.
[19] Fass, D.M., Butler, J.E., Goodman, R.H., 2003. Deacetylase activity is required for deacetylation of histone H3 when cAMP-receptor protein (CRP) activates the adenylate cyclase in PC12 cells. Journal of Biological Chemistry 278(44):43014—43019. http://dx.doi.org/10.1074/jbc.M305905200.
[20] Ling, C., Groop, L., 2009. Epigenetics: a molecular link between environmental factors and type 2 diabetes. Diabetes 58(12):2718—2725. http://dx.doi.org/10.2337/db10-1005.
[21] Parrà, M., 2015. Class IIa HDACs — new insights into their functions in physiology and pathology. FEBS Journal 282(9):1736—1744. http://dx.doi.org/10.1111/febs.13061.
[22] West, A.C., Johnstone, R.W., 2014. New and emerging HDAC inhibitors for cancer treatment. The Journal of Clinical Investigation 124(1):30—39. http://dx.doi.org/10.1172/JCI69378.
[23] Christensen, D.P., Dahllof, M., Lundh, M., Rasmussen, D.N., Nielsen, M.D., Biliestrup, N., et al., 2011. Histone deacetylase (HDAC) inhibition as a novel treatment for diabetes mellitus. Molecular Medicine 17(5—6):378—390. http://dx.doi.org/10.2119/mmed.2011.00021.
[24] Funato, H., Oda, S., Yokofujita, J., Igarashi, H., Kuroda, M., 2011. Fasting and high-fat diet alter histone deacetylase expression in the medial hypothalamus. PLoS One 6(4):e18950. http://dx.doi.org/10.1371/journal.pone.0018950.
