Global differences in specific histone H3 methylation are associated with overweight and type 2 diabetes

Åsa Jufvas, Simon Sjödin, Kim Lundqvist, Risul Amin, Alexander V Vener and Peter Strålfors*

Abstract

Background: Epidemiological evidence indicates yet unknown epigenetic mechanisms underlying a propensity for overweight and type 2 diabetes. We analyzed the extent of methylation at lysine 4 and lysine 9 of histone H3 in primary human adipocytes from 43 subjects using modification-specific antibodies.

Results: The level of lysine 9 dimethylation was stable, while adipocytes from type 2 diabetic and non-diabetic overweight subjects exhibited about 40% lower levels of lysine 4 dimethylation compared with cells from normal-weight subjects. In contrast, trimethylation at lysine 4 was 40% higher in adipocytes from overweight diabetic subjects compared with normal-weight and overweight non-diabetic subjects. There was no association between level of modification and age of subjects.

Conclusions: The findings define genome-wide molecular modifications of histones in adipocytes that are directly associated with overweight and diabetes, and thus suggest a molecular basis for existing epidemiological evidence of epigenetic inheritance.

Keywords: Epigenetic, Histone methylation, Human, Obesity, Overweight, Primary mature adipocytes, Type 2 diabetes

Background

The dynamics of chromatin regulate access to DNA and are therefore under tight control by the host cell and by external stimuli. Reversible covalent post-transcriptional modifications to histones are considered to form one of the major means by which gene transcription and DNA replication are controlled [1]. Histone modifications have been associated with transcriptional control since the discovery of histone acetylation [2]; hyperacetylated histones are linked to actively transcribed genes [2,3].

Methylation of histone H3 at lysine 4 is associated with sites of active gene transcription [4,5]. High levels of dimethylation and trimethylation (H3K4me2 and H3K4me3) are generally found near promoter regions of DNA. Trimethylation, particularly, is found at transcription start sites, while dimethylation flanks these sites of active genes [6,7]. Enhancers appear to host higher levels of monomethylated lysine 4. Dimethylation of lysine 9 (H3K9me2), on the other hand, is a modification found in heterochromatin throughout silenced genes [7] but is also found in actively transcribed genes [8]. Methylation of histones is a reversible and dynamic process that is catalyzed by specific and general histone methyltransferases and demethylases, which are, in turn, dependent on metabolic coenzymes and thus responsive to changes in energy supply and metabolic status [9].

 Obesity and type 2 diabetes (T2D) are characterized by strong hereditary components in addition to such lifestyle-related factors as overeating and physical inactivity; however, no simple relation to gene variants has been discovered. Conversely, genome-wide association studies have uncovered a number of genes that are associated with increased risks of developing the conditions, but the identified genes are each associated with a very low risk and are widely distributed in the population as a whole [10-13].

It is clear that lifestyle and environmental exposure can cause long-lasting susceptibility or resistance to disease, even in later generations, suggesting non-genetic
memory and inheritance. Epidemiological data and clinical and experimental studies indicate that nutritional conditions during early life can strongly influence later susceptibility to T2D. Epigenetic mechanisms have been used to explain the discovery that the famine experienced by pregnant mothers in the Netherlands in World War II affected the birth weights of their children, and their children’s later development of obesity and impaired glucose tolerance [14-16]. In addition, it was found that different starvation or surfeit experiences by parents and grandparents in Överkalix in northern Sweden during the late nineteenth and early twentieth centuries was associated with different susceptibilities to death from cardiovascular disease or T2D in their offspring [17]. Recently, a study of the whole population of Austria found a massively increased risk of diabetes in people born during or immediately after one of three different famines of the twentieth century [18]. In experimental animal studies, the importance of the intrauterine environment has been demonstrated [19-21], as well as a paternal non-genetic transgenerational inheritance of propensity for obesity and diabetes [22,23]. It has been suggested that methylation of DNA, modifications of histones, and noncoding RNA mediate epigenetic inheritance. Methylation of DNA and histone modifications have been shown to be affected by, for example, body mass index (BMI) [24], age [25], intrauterine environment [26-28], glucose exposure [29,30], and exercise [31].

In this study, we investigated whether there is a relation between overweight or obesity, T2D and genome-wide methylation of histone H3 at lysine 4 and at lysine 9 in isolated mature adipocytes.

**Results**

We analyzed the global extent of H3K4me2, H3K4me3, and H3K9me2 in isolated primary mature adipocytes from subjects who were of normal weight, overweight, or overweight with type 2 diabetes. The extent of methylation was determined by SDS-PAGE and immunoblotting using site- and modification-specific antibodies. The extent of specific methylation was normalized for the total amount of histone H3 in each sample, and all values are the median value of three separate experiments. Hence, the extent of histone H3 methylation is determined as the fractional methylation of histone H3.

The level of H3K4me2 was 37% lower in adipocytes from overweight subjects, whether non-diabetic or with T2D, compared with normal-weight non-diabetic subjects (Figure 1). Moreover, when combining the whole group of overweight (non-diabetic and T2D) subjects, the level of H3K4me2 was significantly lower ($P = 0.009$) than in the adipocytes from the normal-weight subjects (not illustrated).

In contrast, the level of H3K4me3 was 40% higher in adipocytes from overweight subjects with T2D than in normal-weight non-diabetic or overweight non-diabetic subjects (Figure 2).

As an association between epigenetic changes and age can be expected and has indeed been observed [32], we examined whether there was any association between the extent of histone modification and the age of the subjects. However, we found no significant association between the global levels of H3K4 dimethylation or trimethylation in the isolated adipocytes and the age of the corresponding subjects (Figure 3).

In contrast with H3K4-methylation, the level of H3K9me2 was similar in adipocytes from T2D and non-diabetic subjects and was not dependent on donor overweight (Figure 4).

**Discussion**

Our findings reveal large genome-wide differences in the level of specific histone methylation in adipocytes from subjects with overweight or T2D compared with normal-weight and non-diabetic subjects. These differences were not related to the age of the subjects donating the adipocytes. The effects were restricted to H3K4 methylation, which is associated with actively transcribed genes, with no corresponding effects in the heterochromatin-defining H3K9 methylation. It is particularly interesting that overweight and T2D are associated
with changes involving nearly half of the dimethylation and trimethylation levels at H3K4 in the adipocytes. This indicates that a large number of genes might be affected by the changed levels of modifications. The underlying cause of these differences probably originates from differences in activities of one or more of the involved methylases or demethylases, or their control. Histone methylation is a reversible process and we cannot exclude changes during surgical procedures and isolation or incubation of the cells, but our findings nevertheless demonstrate large genome-wide changes in overweight and T2D that are directly related to these specific histone modifications. Since most genetic variants associated with T2D appear to be linked to \( \beta \)-cell function and insulin release [10,11] our findings indicate a potential importance of the adipose tissue in hereditability of T2D. An epigenetic link to overweight and T2D is in line with the epidemiological studies discussed previously [14,15,17,18,26].

H3K4me2 is demethylated by LSD1, a FAD-dependent demethylase [33-35]. Interestingly, it has been shown that LSD1 has an increased expression in adipocytes from high-fat diet-fed mice and that adipose energy-expenditure genes are direct targets of repression by LSD1 [34]. Inhibition of LSD1 increases global H3K4 methylation in P19 embryonal carcinoma cells [36] and lowers the body weight of mice fed a high-fat diet [34]. Histone methyltransferase MLL3 catalyzes methylation of H3K4 [37]. Mice with mutations in the catalytic SET-domain of MLL3 show altered gene expression of a number of metabolic genes in adipose tissue, such as Rbp4 [38], which is associated with insulin resistance in human beings [39,40]. The mutant mice also exhibit an altered phenotype, with less adipose tissue and improved insulin sensitivity compared with control mice [38]. Collectively, these reports demonstrate that modifying the global levels of H3K4 methylation experimentally affects adiposity and sensitivity to insulin. This is further supported by experiments showing that the levels of H3K4me3 in PPAR\( \gamma \) promoters correlate with expression levels of PPAR\( \gamma \) during adipogenesis [41]. Interestingly, H3K9me2 was selectively enriched in the entire PPAR\( \gamma \) locus in 3T3-L1 preadipocytes [42], and the level of H3K9me2 correlated inversely with induction of
PPARγ in both murine and human adipogenesis [42]. However, globally we found no correspondence between levels of H3K9 and H3K4 methylation in the mature adipocytes of normal-weight, overweight, or diabetic individuals.

It may be that histone modifications do not determine sites of active transcription, but rather reinforce the effects of nucleosome binding during transcription, for example, in response to the targeting actions of noncoding RNAs [1]. As such, our findings are indicators of large genome-wide changes in transcriptional activities associated with overweight and diabetes, which may be involved in an epigenetically affected propensity for these common disorders. In the future, it will be interesting to analyze to what extent particular sets of genes are affected in different individuals, who may be of normal weight, overweight, or diabetic.

**Conclusions**

Our findings define extensive genome-wide molecular modifications of histones in adipocytes that are directly associated with overweight and diabetes. Effects were restricted to H3K4 methylation, which is associated with actively transcribed genes, with no corresponding effects in the heterochromatin-defining H3K9 methylation. Changes involved 30% to 40% of the dimethylation and trimethylation levels at H3K4 in the adipocytes, indicating that a large number of genes might be affected by the changed levels of modifications. The findings suggest a molecular basis for existing epidemiological evidence of epigenetic inheritance.

**Methods**

**Subjects**

The study was approved by the Regional Ethics Board at Linköping University and has been carried out in accordance with the declaration of Helsinki; all patients obtained written information and gave their informed approval before surgery. Subcutaneous abdominal fat tissue was obtained during elective surgery on patients at the University Hospital, Linköping and Norrköping. Clinical data are summarized in Table 1.

**Isolation and incubation of adipocytes**

Adipocytes were isolated from adipose tissue samples by collagenase digestion (type 1, Worthington, NJ, USA) in modified Krebs-Ringer solution [43]. Following overnight incubation [44], cells were washed with the modified Krebs-Ringer solution and incubated with 0.1 μM N6-phenylisopropyl adenosine and 2.5 μg/ml adenosine deaminase for 10 min, to control the intracellular concentration of cyclic AMP and establish a standardized level of basal lipolysis [45]. Cells were separated from the medium by centrifugation through dinonyl phthalate and were then immediately dissolved in SDS and β-mercaptoethanol with protease and phosphatase inhibitors, frozen within 10 seconds and thawed in boiling water for further analysis [43].

**SDS-PAGE and immunoblotting**

Proteins were separated by SDS-PAGE (14.5% acrylamide) [46] and transferred to a polyvinylidene difluoride blotting membrane (Immobilon-P, Millipore, MA, USA). The extent of H3K4 and H3K9 methylation was analyzed with antibodies against H3K4me2, H3K4me3, or H3K9me2 (Active Motif, Carlsbad, CA, USA). These antibodies are specific for dimethylation or trimethylation, such that the H3K4me2-specific antibodies do not cross-react with H3K4me3. Membranes were stripped of bound antibodies (2% SDS, 62.5 mM Tris, 100 mM β-mercaptoethanol, 60°C, 30 min) and the amount of histone H3 was determined in each sample with antibodies against histone H3 C-terminus (Active Motif), to calculate the ratio of

**Table 1 Characteristics of participating subjects**

| Normal weight (BMI < 25 kg/m²) | Overweight (BMI > 25 kg/m²) | T2D |
|-------------------------------|-----------------------------|-----|
| Female/male                   | 14/0                        | 19/0| 8/2 |
| Age (years)                   | 64.4 ± 8.7                  | 60.2 ± 11.4 | 55.2 ± 15.2 |
| BMI (kg/m²)                   | 22.4 ± 1.5                  | 34.5 ± 8.3 | 41.4 ± 10.8 |
| Fasting glucose (mmol/l)      | 5.8 ± 1.0                   | 6.2 ± 8.9 | 8.0 ± 0.5 |
| Fasting insulin (pmol/l)      | 73.0 ± 64.0                 | 54.5 ± 34.4 | 112.0 ± 114.2 |

Mean ± SD.
histone H3 methylation to the amount of histone H3. To allow comparison between different gels, a standard sample (a mixture of aliquots from 23 subjects) was run in duplicate on every gel and all samples were normalized against the mean of the standard sample. Antibodies were detected using horseradish peroxidase conjugated IgG secondary antibody (Santa Cruz Biotechnical, Santa Cruz, CA, USA) and ECL-plus (Amersham Biosciences, Little Chalfont, Bucks, UK) using chemiluminescence imaging (LAS 1000; Image Gauge v.3.0, Fuji, Tokyo, Japan). Linearity of the antibodies’ responses was ascertained (Additional file 1: Figure S1) and the amounts of each sample subjected to SDS-PAGE were adjusted to fall within this linear range. For the calculations, the median of three separate immunoblottings was used for each of the 43 subjects. Groups were compared with two-tailed Student’s t test, using GraphPad Prism v.5.00 (GraphPad software Inc., San Diego, CA, USA).

**Additional file**

**Additional file 1: Figure S1.** Linearity of immunoblotting with antibodies against H3K4me2, H3K4me3, H3K9me2, and H3 C-terminus.

**Abbreviations**

au: Arbitrary units; BMI: Body mass index; H3K4me2: Histone H3 dimethylated at lysine 4; H3K4me3: Histone H3 trimethylated at lysine 4; H3K9me2: Histone H3 dimethylated at lysine 9; IgG: Immunoglobulin G; SEM: Standard error of the mean; T2D: Type 2 diabetes; SD: Standard deviation.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

ÅJ, AV, and PS conceived and designed the experiments. ÅJ, SS, KL, and RA performed the experiments. ÅJ, SS, KL, AV, and PS analyzed the data. ÅJ, AVV, and PS conceived and designed the experiments. ÅJ, SS, KL, and RA performed the experiments. ÅJ, AVV, and PS wrote the paper. All authors read and approved the final manuscript.

**Acknowledgements**

This work was supported by Swedish Research Council grants to PS and AV, and by Swedish Diabetes Fund and Novo Nordic Fund grants to PS.

**Received:** 1 May 2013 **Accepted:** 12 July 2013 **Published:** 3 September 2013

**References**

1. Zentner GE, Henikoff S: Regulation of nucleosome dynamics by histone modifications. Nat Struct Mol Biol 2013, 20:259–266.

2. Phillips DP: The presence of acetyl groups in histones. Biochem J 1961, 78:258–263.

3. Pogo BG, Alfrey VG, Vinsky AE: RNA synthesis and histone acetylation during the course of gene activation in lymphocytes. Proc Natl Acad Sci USA 1966, 55:805–812.

4. Orford K, Khachemou P, Lai W, Diao MC, Worhunsky DJ, Ferro A, Janzen V, Park PJ, Scadden DT: Differential H3K4 methylation identifies developmentally poised hematopoietic genes. Dev Cell 2008, 14:796–809.

5. Kouzarides T: Chromatin modifications and their function. Cell 2007, 128:693–705.

6. Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K: High-resolution profiling of histone methylations in the human genome. Cell 2007, 129:823–837.

7. Wang Z, Schones DE, Zhao K: Characterization of human epigenomes. Curr Opin Genet Dev 2009, 19:127–134.

8. Zhang Y, Reinberg D: Transcription regulation by histone methylation: Interplay between different covalent modifications of the core histone tails. Gen Dev 2001, 15:2393–2396.

9. Teperino R, Schoorjans K, Ausiello J: Histone methyl transferases and demethylases: can they link metabolism and transcription? Cell Metab 2010, 12:321–327.

10. Doria A, Patti ME, Kahn CR: The emerging genetic architecture of type 2 diabetes. Cell Metab 2008, 8:186–200.

11. Billings UK, Florez JC: The genetics of type 2 diabetes: what have we learned from GWAS? Ann New York Acad Sci 2010, 1212:59–77.

12. Drong AW, Lindgren CM, McCarthy MI: The genetic and epigenetic basis of type 2 diabetes and obesity. Clin Pharmacol Ther 2012, 92:707–715.

13. Sandholt CH, Hansen T, Pedersen O: Beyond the fourth wave of genome-wide obesity association studies. Nutr Diabetes 2012, 2:e37.

14. Ravelli AC, van der Meulen JH, Michels RP, Osmond C, Barker DJ, Hales CN, Barker DJ: Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci USA 2008, 105:17046–17049.

15. Kadi G, Bygren LO, Edvinsson S: Cardiovascular and diabetes mortality determined by nutrition during parents’ and grandparents’ slow growth period. Eur J Hum Genet 2002, 10:682–688.

16. Thurner S, Klimek P, Duftschmid G, Endel G, Kautzky-Willer A, Kasper DC: Quantification of excess risk for diabetes for those born in times of hunger, in an entire population of a nation, across a century. Proc Natl Acad Sci USA 2013, 110:4703–4707.

17. Jousse C, Parry L, Lambert-Langlais S, Maurin AC, Averous J, Bruhat A, Carraro V, Tost J, Letenon P, Chen P, Jockers R, Launay JM, Mallet J, Faoutouche P: Perinatal undernutrition affects the methylation and expression of the leptin gene in adults: implication for the understanding of metabolic syndrome. PASEB J 2011, 25:3271–3278.

18. Raychaudhuri N, Raychaudhuri S, Thamotharan M, Devaakar SJ: Histone code modifications repress glucose transporter 4 expression in the intrauterine growth-restricted offspring. J Biol Chem 2008, 283:13611–13626.

19. Seki Y, Williams L, Vuyk PN, Charron MJ: Minireview. Epigenetic programming of diabetes and obesity: animal models. Endocrinology 2012, 153:1031–1038.

20. Ng SF, Lin CY, Laybutt DR, Barres R, Owens JA, Morris MJ: Chronic high-fat diet in fathers programs β-cell dysfunction in female rat offspring. Nature 2010, 467:963–966.

21. Carone BR, Fauquier L, Habib N, Shea JM, Hart CE, Li R, Bock C, Li C, Gu H, Zamore PD, Meissner A, Jones E, Cho P, Jockers R, Launay JM, Mallet J, Faoutouche P: Perinatal undernutrition affects the methylation and expression of the leptin gene in adults: implication for the understanding of metabolic syndrome. PASEB J 2011, 25:3271–3278.

22. Feinberg AP, Irazuy RA, Fradin D, Ayee MJ, Murakami P, Aspulding T, Eiriksdottir G, Harris TB, Launer L, Gudnason V, Fallin MD: Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. Cell 2010, 143:1094–1096.

23. Finegan AP, Irazuy RA, Fradin D, Ayee MJ, Murakami P, Aspulding T, Eiriksdottir G, Harris TB, Launer L, Gudnason V, Fallin MD: Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. Cell 2010, 143:1094–1096.
cooperativity of histone methylase and demethylase enzymes associated with gene-activating epigenetic marks that co-exist on the lysine tail.

Diabetes 2009, 58:1229–1236.

30. El-Osta A, Braicchio D, Yao D, Poci A, Jones PL, Roeder RG, Cooper ME, Brownlee M: Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. J Exp Med 2008, 205:2409–2417.

31. Barrès R, Yan J, Egan B: Treebak Jonas T, Rasmussen M, Fritz T, Caidahl K, Krook A, O’Gorman Donal J, Zierath Juleen R: Acute exercise remodels promoter methylation in human skeletal muscle. Cell Metab 2012, 15:405–411.

32. Feinberg AP: Epigenetics at the epicenter of modern medicine. J Am Med Assoc 2006, 299:1345–1350.

33. Fang R, Barbera AJ, Xu Y, Rutenberg M, Leonor T, Bi Q, Lan F, Mei P, Yuan GC, Liu C, Peng J, Cheng D, Sui G, Kaiser UB, Shi Y, Shi YG: Human LSD2/ KDM1b/AOF1 regulates gene transcription by modulating intragenic H3K4me2 methylation. Mol Cell 2010, 39:222–233.

34. Hino S, Sakamoto A, Nagaoka K, Anan K, Wang Y, Mimasu S, Umehara T, Yokoyama S, Kosiak K, Nakao M: FAD-dependent lysine-specific demethylase-1 regulates cellular energy expenditure. Nat Commun 2012, 3:758.

35. Shi Y, Lan F, Matson C, Mulligan P, Whetstone JR, Cole PA, Casero RA, Shi Y: Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell 2004, 119:941–953.

36. Lee MK, Wynder C, Schmidt DM, McCafferty DG, Shiekhattar R: Histone H3 lysine 4 demethylation is a target of nonselective antidepressive medications. Chem Biol 2006, 13:563–567.

37. Shilatifard A: Molecular implementation and physiological roles for histone H3 lysine 4 (H3K4) methylation. Curr Opin Cell Biol 2008, 20:341–348.

38. Lee J, Saha PK, Yang QH, Lee S, Park JY, Suh Y, Lee SK, Chen L, Roeder RG, Lee JW: Targeted inactivation of MLL3 histone H3-Lys-4 methyltransferase activity in the mouse reveals vital roles for MLL3 in adipogenesis. Proc Natl Acad Sci USA 2008, 105:19229–19234.

39. Yang Q, Graham TE, Mody N, Prentire F, Peroni OD, Zabolotny JM, Kotani K, Quadro L, Kahn BB: Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 2003, 436:356–362.

40. Öst A, Danielsson A, Liddén M, Eriksson U, Nyström FH, Strålfors P: Retinol-binding protein-4 attenuates insulin-induced phosphorylation of IRS1 and ERK1/2 in primary human adipocytes. FASEB J 2007, 21:3696–3704.

41. Mikkelsen TS, Xu Z, Zhang X, Wang L, Grimmel JM, Lander ES, Rosen ED: Comparative epigenomic analysis of murine and human adipogenesis. Cell 2010, 143:156–169.

42. Wang L, Xu S, Lee J-E, Baldridge A, Grullon S, Peng W, Ge K: Histone H3K9 methyltransferase G9a represses PPARgamma expression and adipogenesis. EMBO J 2013, 32:45–59.

43. Strålfors P, Honnor RC: Insulin-induced dephosphorylation of hormone-sensitive lipase. Correlation with lipolysis and cAMP-dependent protein kinase activity. Eur J Biochem 1989, 182:379–385.

44. Danielsson A, Öst A, Lystedt E, Kjolhede P, Gustavsson J, Nyström FH, Strålfors P: Insulin resistance in human adipocytes occurs downstream of IRS1 after surgical cell isolation but at the level of phosphorylation of IRS1 in type 2 diabetes. FEBS J 2005, 272:141–151.

45. Honnor RC, Dhillion GS, Londos C: cAMP-dependent protein kinase and lipolysis in rat adipocytes. I. Cell preparation, manipulation, and predictability in behavior. J Biol Chem 1985, 260:15122–15129.

46. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970, 227:680–685.

doi:10.1186/1868-7083-5-15
Cite this article as: Jufvas et al.: Global differences in specific histone H3 methylation are associated with overweight and type 2 diabetes. Clinical Epigenetics 2013 5:15.