Body mass index is associated with epigenetic age acceleration in the visceral adipose tissue of subjects with severe obesity

Juan de Toro-Martín1,2, Frédéric Guénard1,2, André Tchernof2,3, Frédéric-Simon Hould4, Stéfane Lebel4, François Julien4, Simon Marceau4 and Marie-Claude Vohl1,2*

Abstract

Background: There is solid evidence that obesity induces the acceleration of liver epigenetic aging. However, unlike easily accessible blood or subcutaneous adipose tissue, little is known about the impact of obesity on epigenetic aging of metabolically active visceral adipose tissue (VAT). Herein, we aimed to test whether obesity accelerates VAT epigenetic aging in subjects with severe obesity.

Results: A significant and positive correlation between chronological age and epigenetic age, estimated with a reduced version of the Horvath’s epigenetic clock, was found in both blood (r = 0.78, p = 9.4 × 10^-12) and VAT (r = 0.80, p = 1.1 × 10^-12). Epigenetic age acceleration, defined as the residual resulting from regressing epigenetic age on chronological age, was significantly correlated with body mass index (BMI) in VAT (r = 0.29, p = 0.037). Multivariate linear regression analysis showed that, after adjusting for chronological age, sex and metabolic syndrome status, BMI remained significantly associated with epigenetic age acceleration in VAT (beta = 0.15, p = 0.035), equivalent to 2.3 years for each 10 BMI units. Binomial logistic regression showed that BMI-adjusted epigenetic age acceleration in VAT was significantly associated with a higher loss of excess body weight following biliopancreatic diversion with duodenal switch surgery (odds ratio = 1.21; 95% CI = 1.04–1.48; p = 0.03).

Conclusions: Epigenetic age acceleration increases with BMI in VAT, but not in blood, as previously reported in liver. These results suggest that obesity is associated with epigenetic age acceleration of metabolically active tissues. Further studies that deepen the physiological relevance of VAT epigenetic aging will help to better understand the onset of metabolic syndrome and weight loss dynamics following bariatric surgery.

Keywords: Epigenetic aging, Obesity, Visceral adipose tissue, Weight loss, Metabolic syndrome, Bariatric surgery, Epigenetic clock

Background

Obesity is a condition with a complex and heterogenous metabolic phenotype [1, 2]. Apart from its main feature, an excessive body fat accumulation in body fat depots, a plethora of additional harmful metabolic disturbances may appear in patients affected by obesity [3]. Among these, increased fasting plasma triglyceride and glucose levels, reduced HDL-cholesterol and elevated blood pressure, together with increased waist circumference, represent a set criteria often used to identify patients with the metabolic syndrome [4]. The frequency and severity of these comorbidities varies widely among patients with obesity, raising questions about the onset of metabolically unhealthy phenotypes in certain patients [5]. Mounting evidence suggests that, in addition to body mass index (BMI), sex, age or body fat distribution, the prevalence of obesity comorbidities depends on the
concurrency of multiple factors, among which genetics and epigenetics would be playing a prominent role [6,7].

Focusing on epigenetics, we and others have shown that altered DNA methylation in obesity would be associated to increased prevalence of metabolic comorbidities [8–13]. As such, both global methylation differences observed in blood and tissue-specific methylation alterations have been found to be associated with healthy or unhealthy obesity phenotypes [14,15]. Likewise, given the innate plasticity of DNA methylation at cytosine-phosphate-guanine (CpG) dinucleotides [16], its modulations have been found to be associated with healthy or unhealthy obesity phenotypes [14,15].

The concept of epigenetic aging emerges as a straightforward idea, given that altered DNA methylation in obesity would be associated to increased prevalence of metabolic comorbidities [8–13]. As such, both global methylation differences observed in blood and tissue-specific methylation alterations have been found to be associated with healthy or unhealthy obesity phenotypes [14,15]. Likewise, given the innate plasticity of DNA methylation at cytosine-phosphate-guanine (CpG) dinucleotides [16], its modulations have been found to be associated with healthy or unhealthy obesity phenotypes [14,15].

The concept of epigenetic aging emerges as a straightforward idea, given that altered DNA methylation in obesity would be associated to increased prevalence of metabolic comorbidities [8–13]. As such, both global methylation differences observed in blood and tissue-specific methylation alterations have been found to be associated with healthy or unhealthy obesity phenotypes [14,15]. Likewise, given the innate plasticity of DNA methylation at cytosine-phosphate-guanine (CpG) dinucleotides [16], its modulations have been found to be associated with healthy or unhealthy obesity phenotypes [14,15].

Given the relevance of liver as a central regulator of metabolism under both physiological and pathological conditions, these results point out to a specific impact of obesity on the epigenetic aging of metabolically active tissues [25]. These findings gain significance when considering that the acceleration of epigenetic age in visceral adipose tissue (VAT), a key tissue in obesity development and progression, has never been analyzed, probably due to its inaccessibility, as compared to more accessible blood or subcutaneous adipose tissue.

| Variable                  | Study participants (n = 52) | Liver (n = 62) | Liver (obesity) (n = 40) |
|---------------------------|----------------------------|---------------|-------------------------|
| Mean SD                   | Mean SD                    | Mean SD       | Mean SD                 |
| Men (n = 24)              | (n = 17)                   | (n = 16)      | (n = 6)                 |
| Women (n = 28)            | (n = 45)                   | (n = 24)      | (n = 34)                |
| Chronic age               | 35.0 10.6                  | 55.6 17.3     | 51.2 10.0               |
| BMI                       | 54.4 9.1                   | 35.9 14.4     | 54.1 4.7                |

Data are expressed as mean and standard deviation (SD). Liver and liver (obesity) refer to publicly available data (GSE48325) [27] and a subset of subjects with BMI > 40 kg/m², respectively. p stands for p values obtained in Student’s t test. BMI body mass index.
similar to those previously obtained in liver [24], and successfully replicated herein with our reduced version of the epigenetic clock ($r = 0.89$, $p = 3.9 \times 10^{-22}$) (Fig. 1c), as well as to those in the liver of subjects with obesity ($r = 0.87$, $p = 3.3 \times 10^{-13}$) (Fig. 1d). Residuals resulting from regressing epigenetic age on chronological age were then used as a measurement of epigenetic age acceleration, whose association with BMI was tested. Epigenetic age acceleration in blood was not correlated with BMI ($r = 0.21$, $p = 0.14$) (Fig. 1e), as previously reported [24]. In contrast, a significant and positive correlation was found between epigenetic age acceleration and BMI in VAT ($r = 0.29$, $p = 0.037$) (Fig. 1f). A positive correlation was also observed in liver ($r = 0.40$, $p = 0.0013$) (Fig. 1g), where we were able to consistently reproduce previously reported experimental findings [24]. Results in the validation dataset showed that BMI correlated with epigenetic age acceleration in the liver of subjects with severe obesity to a similar extent to what we observed in VAT ($r = 0.33$, $p = 0.038$) (Fig. 1h).

In view that results from multivariate regression models, adjusted by chronological age, sex and metabolic syndrome, confirmed that epigenetic age acceleration was not dependent on BMI in blood ($\beta = 0.16; 95\% CI = -0.04\,\text{--}\,0.36; p = 0.12$) (Table 2). Our results also showed that BMI remained significantly associated with epigenetic age acceleration in VAT ($\beta = 0.15; 95\% CI = 0.04\,\text{--}\,0.28; p = 0.03$) (Table 2), as well as in liver ($\beta = 0.16; 95\% CI = 0.07\,\text{--}\,0.25; p = 5.6 \times 10^{-9}$) and in the liver of subjects with obesity ($\beta = 0.24; 95\% CI = 0.04\,\text{--}\,0.45; p = 0.02$). With beta estimates representing the acceleration of epigenetic age in years by unit change of BMI and after correcting for chronological age, the observed epigenetic age acceleration in VAT was equivalent to 2.20 years for each 10 BMI units, similar to the additional 2.28 years observed in liver, and the 3.04 years in the liver of subjects with obesity (Table 2).

Epigenetic age acceleration of VAT correlates with BMI only in men
In view that results from multivariate regression models showed a significant association between sex and epigenetic
| Variable                  | Blood            | Blood (Visceral adipose tissue) | Liver | Liver (Obesity) |
|---------------------------|------------------|-------------------------------|-------|----------------|
|                           | β   | 95% CI     | p       | β    | 95% CI     | p       | β    | 95% CI     | p       | β    | 95% CI     | p       |
| Chronological age         | 0.87 | 0.67 – 1.07   | 1.48 × 10⁻¹¹ | 0.66 | 0.53 – 0.80   | 2.63 × 10⁻¹³ | 0.71 | 0.62 – 0.81   | < 2 × 10⁻⁶ | 0.80 | 0.65 – 0.95   | 7.56 × 10⁻¹³ |
| BMI                       | 0.16 | − 0.04 – 0.36 | 0.12       | 0.15 | 0.04 – 0.28   | 0.003     | 0.16 | 0.07 – 0.25   | 5.6 × 10⁻⁴ | 0.24 | 0.004 – 0.45  | 0.02     |
| Sex                       | − 3.11 | − 6.54 – 0.33 | 0.08       | 2.89 | 0.59 – 5.20   | 0.002     | − 1.47 | − 4.07 – 1.13 | 0.26     | − 0.28 | − 4.12 – 3.56 | 0.88     |
| Metabolic syndrome        | 0.47 | − 2.75 – 3.69 | 0.77       | 1.42 | − 0.74 – 3.58 | 0.19      | −      | −          | −       | −      | −          | −       |
| Adjusted R²               | 0.64 | −           | −          | 0.67 | −           | −          | 0.82  | −          | −       | 0.78  | −          | −       |
| Age acceleration          | 1.83 | −           | −          | 2.20 | −           | −          | 2.28  | −          | −       | 3.04  | −          | −       |

Estimates (β), 95% confidence intervals (95%CI), and p values (p) are from multivariate linear regression models of DNA methylation age acceleration adjusted by chronological age, body mass index (BMI), sex and metabolic syndrome. β represents the acceleration of epigenetic age in years by unit change of dependent variable. Age acceleration stands for the increase of epigenetic age in years for each 10-point increase in BMI.
age acceleration (Table 2), the latter was compared between men and women, and its correlation with BMI was analyzed separately. Significant sex differences were observed in blood, with men having a higher epigenetic age acceleration than women (2.1 vs −1.8, p = 0.02) (Fig. 2a). However, BMI was not correlated with epigenetic age acceleration in blood, neither in men (r = 0.33, p = 0.12) (Fig. 2b) nor in women (r = 0.03, p = 0.89) (Fig. 2c). Although no difference was found in epigenetic age acceleration between men and women in VAT (−1.11 vs 0.95, p = 0.07) (Fig. 2d), BMI was significantly and positively correlated with epigenetic age acceleration in men (r = 0.42, p = 0.04) (Fig. 2c), but not in women (r = 0.19, p = 0.35) (Fig. 2d). With a population of 24 men and 28 women, Pearson correlation coefficients larger than 0.5 are needed to attain a statistical power of 0.8. By contrast, a significant correlation between BMI and liver epigenetic age acceleration was observed only in women (r = 0.45, p = 0.002), but not in men (r = 0.38, p = 0.13). Similar results were found in the liver obesity group in both women (r = 0.42, p = 0.013) and men (r = −0.05, p = 0.93) (Additional file 1: Figure S2).

Epigenetic age acceleration of VAT is associated with postsurgery weight loss

The measurement of epigenetic age acceleration adjusted by chronological age, sex and BMI in blood and VAT was not significantly correlated with each other (Fig. 3a). Binomial logistic regression was further used to test whether adjusted epigenetic age acceleration was associated with metabolic syndrome and/or with weight loss trajectory groups. On the one hand, results from the linear trend test did not show an association between the adjusted epigenetic age acceleration with metabolic syndrome, neither in blood (OR = 1.02; 95% CI = 0.92–1.13; p = 0.76) (Fig. 3b) nor in VAT (OR = 1.11; 95% CI = 0.96–1.31; p = 0.18) (Fig. 3c). On the other hand, weight loss clustering procedure resulted in three trajectory groups depending on the percentage of excess body weight loss (%EBWL) as follows: normal weight loss (NWL), intermediate weight loss (IWL), and low weight loss (LWL), representing 65%, 30%, and 5% of patients (Fig. 3d). Patients from IWL and LWL groups were reassigned into a unique group (ILWL). While no association was found in blood (OR = 1.01; 95% CI = 0.90–1.12; p = 0.91) (Fig. 3e), the probability of belonging to the NWL group significantly increased with the adjusted epigenetic age acceleration of VAT (OR = 1.21; 95% CI = 1.04–1.48; p = 0.03) (Fig. 3f). In other words, patients showing higher epigenetic age acceleration in VAT exhibited a more pronounced weight loss response to bariatric surgery. Whether the adjusted epigenetic age acceleration was associated with metabolic syndrome

---

**Fig. 2** The acceleration of epigenetic aging in VAT correlates with BMI in men. Panels a and d compare the distribution of DNA methylation (DNAm) age acceleration, defined as the residual from regressing DNAm age on chronological age, between men and women in blood (a) and visceral adipose tissue—VAT—(d). Boxplots represent the distribution of phenotype data with median (dark horizontal line) and interquartile range (box), and p stands for the p values obtained in Student’s t test for independent samples. The correlation between DNAm age acceleration and body mass index (BMI) in blood (b and c, red dashed line) and VAT (e and f, yellow dashed line) is shown separately in men and women. Residuals over zero (horizontal black line) stand for an acceleration of DNAm age, while r and p refer to Pearson correlation coefficients and p values, respectively. Blue and gray dots refer to men and women, respectively.
and/or with weight loss trajectory groups was further tested separately in men and women with no significant result (data not shown).

**Discussion**

This is, to our knowledge, the first study to show the association between BMI and epigenetic age acceleration of VAT. Concretely, the main finding of the present work revealed that increasing BMI in obesity is positively correlated with epigenetic age acceleration in VAT. Importantly, this study also replicates the absence of association between BMI and epigenetic age acceleration in blood [24]. The present results also revealed that VAT epigenetic aging is more strongly related to BMI in men, as compared to women. Additional findings suggested that VAT epigenetic age acceleration may not have a major association with features of the metabolic syndrome in obesity, but a potential and significant effect on the evolution of body weight loss following bariatric surgery.

Previously, Horvath et al. [24] carried out an elegant study to test whether obesity may increase the acceleration of tissue aging. The original version of the epigenetic clock [21], herein used in its reduced form, was utilized to test whether BMI correlated with epigenetic age acceleration in various tissues. Results were very enlightening since they suggested a tissue-specific effect of obesity on the acceleration of epigenetic aging. Concretely, BMI was highly correlated with accelerated liver aging, whereas no effect was observed in blood and, more importantly, nor in subcutaneous adipose tissue. Since fat depots are submitted to an important metabolic stress during weight gain, we found particularly interesting and paradoxical the fact that epigenetic aging of subcutaneous adipose tissue was not altered by increasing BMI, and we decided to investigate whether a distinct effect of obesity on VAT was taking place. We hypothesized that BMI relates to accelerated epigenetic aging of metabolically active tissues, such as VAT and liver, and that such accelerated aging may in part be responsible for the shifting from healthy to unhealthy obesity phenotypes. Although the latter part of the hypothesis was not fully supported by results, our findings are still of interest. In particular, our results support a specific impact of obesity on the epigenetic aging of key metabolic tissues. As mentioned...
above, the significant correlation showed herein between BMI and epigenetic age acceleration in VAT mirrored in some way that previously observed in liver [28]. Both the similar effect size of BMI on epigenetic age acceleration and the estimated epigenetic aging in years found in both VAT and liver further supported the hypothesis of a tissue-specific dysregulation of methylation [15, 29, 30].

Some methodological differences between the previous study in the liver and the present analysis need to be clearly pointed out. First, we had to use a reduced version of the Horvath’s clock due to technical constraints. However, the successful replication of previous results supported the use of our modified version of the epigenetic clock. Concretely, almost identical correlation coefficients between chronological and epigenetic age were observed in both studies, while correlation coefficients with BMI in liver showed the same magnitude and direction than in Horvath’s study [24]. Second, unlike in the liver study, all the patients from the present work had severe obesity, with or without associated metabolic disturbances, which somehow limited the BMI study range. Nevertheless, the correlation between BMI and epigenetic age acceleration in VAT and liver from patients with obesity was again highly similar, thus supporting the validity of this study. Second, the decision of matching pairs for age, BMI and metabolic syndrome within each sex further limited the spectrum of eligible participants, leading to a narrower range of age, as compared to the Horvath’s study. Both BMI and age ranges may influence the final results, as well as sex representation [28, 31]. Our results showed that men had higher epigenetic aging rates than women in blood, which has been previously linked to a lower morbidity but higher mortality in men than in women [28]. On the other hand, while the impact of BMI on liver epigenetic aging has been previously observed in men and women [24], herein association in VAT was seen only in men. Though, the correlation between BMI and epigenetic age acceleration in liver was only significant in women in the discovery dataset in [24]. Herein, we also observed a significant correlation between BMI and liver epigenetic age acceleration only in women, which may suggest a different impact of BMI on liver epigenetic aging between men and women. Although the present results did not show a significant association between the adjusted measurement of epigenetic age acceleration and the presence or absence of metabolic syndrome, these results still suggest a sex-specific impact of obesity on VAT epigenetic aging, which could explain, in part, the distinct development of obesity comorbidities between men and women, rather than waist circumference or visceral fat accumulation per se [32]. In any case, these results should be taken with caution, because the effect sizes observed, together with the number of samples from each sex, could lead to uncertain results. We acknowledged that this represents a limitation of the present study. Similarly, the level of statistical power achieved in the entire dataset does not allow us to derive statements regarding the association between BMI and epigenetic aging as consistent as desired. This fact, together with the heterogeneous and inconsistent results previously reported in blood [22, 24, 31], prevent us from ascertaining an actual lack of association between BMI and the epigenetic age acceleration in blood. In spite of reducing the total number of subjects, we tried to support our findings by reanalyzing blood and VAT samples in a dataset without extreme age values, as previously shown [31]. Interestingly, a stronger relationship between BMI and epigenetic age acceleration in VAT was observed, as well as the near absence of association in blood, supporting results observed in the entire dataset. Yet, further studies in larger cohorts designed to capture a broad BMI and age spectrum, as well as to attain a reasonable degree of statistical power, are still required to elucidate the actual impact of BMI on the epigenetic age acceleration in blood and VAT. Taken together, although the specific nature of our cohort somehow limits the number of participants, we consider it worth to be studied, since the findings drawn from its analysis add valuable insights to the discussion on the role of BMI on epigenetic aging.

As just mentioned, another relevant finding of the present work that deserves to be highlighted is the absence of association between VAT epigenetic aging and metabolic syndrome. This is important since it does not support the second term of the main hypothesis, that is, the development of obesity comorbidities through an acceleration of VAT epigenetic age. Among others, a potential explanation of this result is the complexity of the chosen composite endpoint, that is, the presence or absence of metabolic syndrome defined as the sum of a number of metabolic disturbances [4]. Another rather unexpected result was the positive association between the acceleration of VAT epigenetic aging and postsurgery weight loss trajectories, especially when BMI is negatively associated with the percentage of excess body weight loss following bariatric surgery [26]. However, these latter results may suggest that bariatric surgery would yield more beneficial outcomes in those patients with aged VAT. Previous studies have already reported a deep epigenetic remodeling after different weight loss interventions in adipose tissue [33, 34]. In view of that, these results could imply that a more intense methylation remodeling may be taking place in an aged VAT following bariatric surgery, leading to a tissue rejuvenation. Since our longitudinal weight-loss study is still not finished, it is not yet possible to have access to postsurgery VAT methylation data that would allow us to
establish a causal relationship. It is also worth noting that this is a whole-tissue analysis and that knowing which cell type is responsible for the accelerated aging of VAT might help understand the link with the response to weight loss. In this regard, senescence of adipocytes is a hallmark of adipose tissue aging, and it would be expected that senescent mature adipocytes would mobilize less fat during weight loss [35]. However, the observed accelerated aging might be associated with senescence of another cell type which might impair lipid mobilization. This could be cells from the stromal vascular fraction involved in adipose tissue remodeling [36], e.g. immune cells or endothelial cells of the vascular bed which could reduce blood flow to the tissue [37]. Further studies are in process and will help understand the actual impact of bariatric surgery on VAT epigenetic aging.

Conclusions
In conclusion, our results seem to corroborate that obesity accelerates epigenetic aging of metabolically active tissues, such as VAT and liver. Likewise, these results suggest that epigenetic age acceleration in blood does not correlate with BMI in obesity. Moreover, BMI seems to have a more pronounced effect on epigenetic age acceleration in men than in women. Finally, while not having an effect on metabolic syndrome development, the acceleration of VAT epigenetic aging seems to play an important role in weight loss dynamics following bariatric surgery.

Methods
Study participants
A total of 56 patients, 28 men and 28 women with severe obesity (BMI > 40 kg/m²) and undergoing bariatric surgery (biliopancreatic diversion with duodenal switch) at the Quebec Heart and Lung Institute, were selected to participate in the present study. Patients were matched within each sex for age, BMI and the presence or absence of metabolic syndrome. Omental samples (VAT) were obtained during the course of surgery and blood samples were collected preoperatively. The surgical protocol, blood and VAT sample collection, and the standardized procedures to measure anthropometric and metabolic parameters are described elsewhere [38]. Patients were diagnosed with the metabolic syndrome when three or more criteria of the National Cholesterol Education Program Adult Treatment Panel III guidelines were present [4]. Waist circumference, blood pressure, HDL-cholesterol, plasma triglycerides and fasting glucose levels were measured preoperatively and used to identify those with the metabolic syndrome. Severe obesity was defined as BMI > 40 kg/m², calculated as weight in kilograms divided by height in meters squared. Due to the lack of accurate phenotype data, one patient and its matching pair were excluded from further analyses.

Genome-wide DNA methylation analysis
Genomic DNA of the 56 study participants was extracted from 200 mg of VAT using the DNeasy Blood & Tissue kit (QIAGEN, Mississauga, Ontario, Canada) and isolated from the blood buffy coat using the GenElute™ Blood Genomic DNA kit (Sigma, St Louis, MO, USA). Following quantification of DNA using both NanoDrop Spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and PicoGreen DNA methods, DNA (1 μg) was bisulfite converted and quantitative genome-wide methylation analysis was conducted using Infinium HumanMethylation450 (450k) and EPIC platforms (Illumina, San Diego, CA) interrogating over 485,000 and 850,000 CpG sites at single nucleotide resolution, respectively. Methylation arrays were processed at the McGill University and Génomique Québec Innovation Centre (Montreal, Canada) according to the manufacturer’s instructions (Illumina, San Diego, CA). Methylation data was preprocessed and normalized using the minfi R package [39]. Before background correction and normalization, the 450k and EPIC arrays were combined and integrated into a virtual 450k array, leaving 453,093 CpG sites for further statistical analyses. The single-sample Noob (ssNoob) method was the preferred normalization procedure, as previously recommended when integrating data from multiple Infinium methylation arrays [40]. Methylation levels (beta values; β) were estimated as the ratio of signal intensity of the methylated alleles to the sum of methylated and unmethylated intensity signals of the alleles (β value = C/(T+C)). The β values varied from 0 (no methylation) to 1 (100% methylation).

The overall correlation across 453,093 CpG sites between 450 k and EPIC arrays was very high in blood and VAT (r = 0.992 in both tissues). One sample did not fulfill methylation quality control criteria and was excluded, together with its matching pair, from further analyses.

Epigenetic clock
Epigenetic age was estimated for each patient in VAT and blood according to the Horvath’s epigenetic clock [21], currently the gold-standard for determining epigenetic age in humans [41]. Because part of methylation data was obtained from the EPIC array, which does not include the whole dataset of 353 CpG sites used to build the original epigenetic clock, we estimated epigenetic age by using a reduced version of it, as previously done [42]. The final dataset used to estimate epigenetic age consisted on a set of 336 CpG sites, 4.8% less than in the original dataset. The correlation among CpG sites included in the epigenetic clock was similarly strong in blood (r = 0.992) and VAT (r = 0.991) between 450k and EPIC arrays, with a total of 323 CpG sites in blood
were performed in R (https://www.R-project.org). Regression models and the rest of statistical calculations BMI was set as a continuous variable in logistic regression. In the surgery group, both set as dichotomous variables. Epigenetic metabolic syndrome, as well as into a weight loss trajectory group, a healthy or unhealthy phenotype (presence or absence of epigenetic age acceleration). Binomial logistic regression was further used to test the association between BMI and its correlation with BMI age [43]. Briefly, the traj procedure uses a factor analysis to select nonredundant measurements, followed by a cluster analysis to identify subsets of patients with similar weight loss trajectories [44]. Weight loss data from 46 patients was available for trajectory group assessment. Postsurgery weight was measured during postoperative visits or phone calls a follow-up period of four years, and a total of seven postsurgery time points at 3, 6, 12, 18, 24, 36, and 48 months were used for cluster estimation. Postsurgery weight loss was defined as the percentage of excess body weight loss (%EBWL), calculated as the difference between actual body weight loss (initial BMI minus actual BMI) and ideal body weight loss (initial BMI minus ideal BMI fixed at 25 kg/m²) [45]. Resulting groups allowed the categorization of patients as a function of their %EBWL.

Weight loss trajectories
Post surgery weight loss trajectories were estimated for each participant by identifying clusters of individual longitudinal weight loss data implemented in the traj R package [43]. Briefly, the traj procedure uses a factor analysis to select nonredundant measurements, followed by a cluster analysis to identify subsets of patients with similar weight loss trajectories [44]. Weight loss data from 46 patients was available for trajectory group assessment. Postsurgery weight was measured during postoperative visits or phone calls a follow-up period of four years, and a total of seven postsurgery time points at 3, 6, 12, 18, 24, 36, and 48 months were used for cluster estimation. Postsurgery weight loss was defined as the percentage of excess body weight loss (%EBWL), calculated as the difference between actual body weight loss (initial BMI minus actual BMI) and ideal body weight loss (initial BMI minus ideal BMI fixed at 25 kg/m²) [45]. Resulting groups allowed the categorization of patients as a function of their %EBWL.

Statistics
Phenotype data was checked for normality with the Kolmogorov-Smirnov test. Two-group comparisons were tested with Student’s t-test for paired and independent samples, as appropriate. Epigenetic age acceleration was defined as the residual resulting from regressing epigenetic age on chronological age, and its correlation with BMI was tested in both VAT and blood using Pearson correlation coefficients. A multivariate linear regression model including chronological age, sex and metabolic syndrome was further used to test the association between BMI and epigenetic age acceleration. Binomial logistic regression was used to predict the probability that a patient falls into a healthy or unhealthy phenotype (presence or absence of metabolic syndrome), as well as into a weight loss trajectory group, both set as dichotomous variables. Epigenetic age acceleration adjusted by chronological age, sex and BMI was set as a continuous variable in logistic regression. Regression models and the rest of statistical calculations were performed in R (https://www.R-project.org) [46]. Power calculations were performed in G*Power [47].

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s13148-019-0754-6.

Additional file 1: Table S1. Data summary of participants in the study cohort. Figure S1. The acceleration of epigenetic aging in VAT correlates with BMI in middle-aged subjects. Figure S2. The acceleration of epigenetic aging in liver correlates with BMI in women. Figure S3. Correlation among CpG sites included in the epigenetic clock between 450k and EPIC arrays.

Abbreviations
%EBWL: Percentage of excess body weight loss; BMI: Body mass index; CpG: Cytosine-phosphate-guanine dinucleotides; DNAm age: DNA methylation age; IWL: Intermediate weight loss; LWL: Low weight loss; NWL: Normal weight loss; VAT: Visceral adipose tissue

Acknowledgements
We thank all participants for their collaboration, and the surgeons of the Bariatric Surgery Center of the Quebec Heart and Lung Institute (Odette Lescelleur, Laurent Biertho, Annie Lafontaine) for their invaluable collaboration in clinical care and patient recruitment. We are grateful to Paule Marceau for data management, Catherine Raymond for DNA extraction and technical assistance, and the staff of the Quebec Heart and Lung Institute Biobank for patient consent and sample processing. We also sincerely thank our long-term collaborator Picard Marceau, with whom this project was initiated.

Authors’ contributions
JTM performed statistical analysis, interpreted the data and wrote the manuscript; MCV and FG conceived and designed the research; AT participated in the elaboration of the study design and critically reviewed the manuscript. FSH, SL, FJ and SM participated in clinical care, patient recruitment, and tissue sampling. All authors read and approved the final manuscript.

Funding
This study was supported by a grant-in-aid from the Heart and Stroke Foundation of Canada (G-17-0016627) and by the Canada Research Chair in Genomics Applied to Nutrition and Metabolic Health, held by MCV. JTM received a postdoctoral fellowship from the Fonds de Recherche de Quebec-Santeé.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
This study was approved by Laval University and Quebec Heart and Lung Institute ethics committees and was performed in accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent.

Consent for publication
Not applicable.

Competing interests
AT receives research funding from Johnson & Johnson Medical companies, Pfizer and Medtronic for studies on bariatric surgery. The rest of authors declare that they have no competing interests.

Author details
1Institute of Nutrition and Functional Foods (INAF), Université Laval, Pavillon des Services (2729 K), 2440, boul. Hochelaga, Quebec, QC G1V 0A6, Canada. 2School of Nutrition, Université Laval, Quebec, QC, Canada. 3Quebec Heart and Lung Institute Research Center, Quebec, QC, Canada. 4Department of Surgery, Université Laval, Quebec, QC, Canada.
45. Marceau P, Biron S, Marceau S, Hould F-S, Lebel S, Lescelleur O, et al. Long-term metabolic outcomes 5 to 20 years after biliopancreatic diversion. Obes Surg. 2015;25(9):1584–93.

46. R Core Team. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2018.

47. Faul F, Erdfelder E, Lang A-G, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007;39(2):175–91.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.