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# *LINE-1* methylation in visceral adipose tissue of severely obese individuals is associated with metabolic syndrome status and related phenotypes

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## Abstract

**Background:** Epigenetic mechanisms may be involved in the regulation of genes found to be differentially expressed in the visceral adipose tissue (VAT) of severely obese subjects with (MetS+) versus without (MetS-) metabolic syndrome (MetS). Long interspersed nuclear element 1 (*LINE-1*) elements DNA methylation levels (% meth) in blood, a marker of global DNA methylation, have recently been associated with fasting glucose, blood lipids, heart diseases and stroke.

**Aim:** To test whether *LINE-1*%meth levels in VAT are associated with MetS phenotypes and whether they can predict MetS risk in severely obese individuals.

**Methods:** DNA was extracted from VAT of 34 men (MetS-:  $n = 14$ , MetS+:  $n = 20$ ) and 152 premenopausal women (MetS-:  $n = 84$ ; MetS+:  $n = 68$ ) undergoing biliopancreatic diversion for the treatment of obesity. *LINE-1*%meth levels were assessed by pyrosequencing of sodium bisulfite-treated DNA.

**Results:** The mean *LINE-1*%meth in VAT was of 75.8% (SD = 3.0%). Multiple linear regression analyses revealed that *LINE-1*%meth was negatively associated with fasting glucose levels ( $\beta = -0.04$ ;  $P = 0.03$ ), diastolic blood pressure ( $\beta = -0.65$ ;  $P = 0.03$ ) and MetS status ( $\beta = -0.04$ ;  $P = 0.004$ ) after adjustments for the effects of age, sex, waist circumference (except for MetS status) and smoking. While dividing subjects into quartiles based on their *LINE-1*%meth (Q1 to Q4: lower %meth to higher %meth levels), greater risk were observed in the first (Q1: odds ratio (OR) = 4.37,  $P = 0.004$ ) and the second (Q2: OR = 4.76,  $P = 0.002$ ) quartiles compared to Q4 (1.00) when adjusting for age, sex and smoking.

**Conclusions:** These results suggest that lower global DNA methylation, assessed by *LINE-1* repetitive elements methylation analysis, would be associated with a greater risk for MetS in the presence of obesity.

**Keywords:** Blood pressure, Epigenetics, Fasting glucose, Global DNA methylation, *LINE-1*, Metabolic syndrome, Severe obesity, Visceral adipose tissue

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## Background

Accumulation of fat preferentially in the abdominal cavity is frequently associated with clustering of metabolic and inflammatory alterations, often referred to as metabolic syndrome (MetS) [1,2]. However, there exists a large heterogeneity in the development of these metabolic complications among obese individuals [3,4], which may partly be explained by impairment of adipose tissue function [5,6]. Previous studies observed differential gene expression patterns in visceral adipose tissue (VAT) of 'metabolically healthy' vs 'metabolically unhealthy' obese individuals [7-9]. It is thus possible that some of these differentially expressed genes may be functionally related to VAT dysfunction and MetS-related phenotypes. Although polymorphisms within several genes have previously been associated with both gene expression variability and MetS components [5], epigenetic mechanisms may also contribute to this variability.

Several epidemiological and animal studies demonstrated that a detrimental environment during fetal and postnatal periods, such as maternal obesity and gestational diabetes, is associated with increased risk of obesity and metabolic complications later in life [10,11]. These long-term complications would partly be explained by impaired organogenesis and cellular energy metabolism [12], as it may be the case regarding adipose tissue development and function [12-14]. Thus, impairments of normal development will ultimately change gene expression patterns in key metabolic pathways involved in obesity and MetS, which may potentially be mediated through epigenetic mechanisms [15,16]. DNA methylation is the most widely studied epigenetic phenomenon in humans. While association analyses between CpG methylation levels of candidate genes and complex diseases are increasing [17], an emerging literature uses CpG methylation of repetitive DNA sequences in disease association analyses [18]. This is the case for long interspersed nuclear element 1 (*LINE-1*) elements, which is a marker of the global DNA methylation of the genome [19]. *LINE-1* elements are a family of transposon-derived sequence repeats dispersed in the genome [20]. These repetitive elements are normally heavily methylated and their hypomethylation is commonly associated with cancer development and progression [21,22].

Interestingly, recent epidemiological studies also identified some associations between *LINE-1* methylation variability and ischemic heart disease and stroke [23], as well as with MetS phenotypes, such as plasma fasting glucose [24] and plasma lipid levels [24,25].

In view of the recent observations regarding blood *LINE-1* methylation levels and MetS-related phenotypes, the aim of the present study was to test whether *LINE-1* methylation levels in VAT are associated with MetS phenotypes and whether they can predict MetS risk in severely obese individuals.

## Methods

### Patient selection

The study subjects were severely obese Caucasian men (MetS-:  $n = 14$ ; MetS+:  $n = 20$ ) and premenopausal women (MetS-:  $n = 84$ ; MetS+:  $n = 68$ ) undergoing a biliopancreatic diversion with sleeve gastrectomy to treat obesity [26] at the Institut Universitaire de Cardiologie et de Pneumologie de Québec (Québec City, Canada) from June 2000 to June 2010, and for whom DNA samples from VAT were available. The study subjects were not taking any medications to treat MetS features and were not affected by type 2 diabetes mellitus [27]. Body weight, height, waist circumference, resting systolic and diastolic blood pressure were measured using standardized procedures [28]. The day of surgery, fasting blood samples were drawn into EDTA-containing tubes and centrifuged for plasma lipid and glucose concentration measurements [28]. VAT from the greater omentum was sampled during the surgery, as previously reported [26,29]. The diagnosis of MetS was based on the International Diabetes Federation (IDF) definition [30]. MetS+ subjects had to be centrally obese (waist circumference  $\geq 88$  cm for women and  $\geq 102$  cm for men; USA cut-off values from the Adult Treatment Panel III [31]) with  $\geq 2$  other MetS criteria among the following: fasting plasma glucose  $\geq 5.6$  mmol/l, high-density lipoprotein (HDL)-cholesterol  $< 1.29$  mmol/l for women and  $< 1.03$  mmol/l for men, triglycerides  $\geq 1.7$  mmol/l, systolic blood pressure (SBP)  $\geq 130$  mmHg or diastolic blood pressure (DBP)  $\geq 85$  mmHg. All subjects provided a written informed consent to participate in this study, which received the approval of Université Laval Ethics Committee.

### Analysis of *LINE-1* CpG methylation in VAT

DNA was extracted from VAT using the DNeasy Blood & Tissue kit (Qiagen, Mississauga, Ontario, Canada), as recommended by the manufacturer. DNA extracts were stored at  $-80^{\circ}\text{C}$  until quantitative methylation analysis using the pyrosequencing technology from Qiagen [32], which was performed by the McGill University and Genome Québec Innovation Center Genotyping Platform team (Montréal, Canada). DNA (1  $\mu\text{g}$ ) was treated with sodium bisulfite followed by a purification step using the EZ-96 DNA Methylation-Gold kit (Zymo Research, Orange, CA, USA). The polymerase chain reaction (PCR) was performed in a 25  $\mu\text{l}$  total volume. The final concentrations were: 0.05 U/ $\mu\text{l}$  for Qiagen HotStarTaq DNA Polymerase with 1.25  $\times$  PCR buffer, plus 1.0 mM of magnesium chloride, 0.2  $\mu\text{M}$  for each primer and 0.50 mM for dNTP mix (Roche NucleoMix). PCR started with an initial denaturation of 15 minutes at  $95^{\circ}\text{C}$  followed by 45 cycles of 20 s at  $95^{\circ}\text{C}$ , 30 s at  $56^{\circ}\text{C}$  and 60 s at  $72^{\circ}\text{C}$ ; the reaction finished with 5 minutes at  $72^{\circ}\text{C}$ . PCR products were purified and sequenced by pyrosequencing as previously described [33] using 0.3  $\mu\text{M}$  sequencing primer. Primer

sequences were previously published [34,35], and are the following: forward, 5'-TTT TGA GTT AGG TGT GGG ATA TA-3'; reverse, 5'-Biotin-AAA ATC AAA AAA TTC CCT TTC-3'; and sequencing, 5'-AGT TAG GTG TGG GAT ATA GT-3'. The targeted region comprised three CpGs located in the 5' region of *LINE-1* elements [19] and had the following bisulfite-treated DNA sequence: 5'-TTC/TGTGGTGC/TGTC/TG-3', where C/T corresponded to methylated (C) and unmethylated (T) cytosine at each CpG site. Since the %meth levels of the three CpG sites were highly correlated between each other ( $r = 0.57 - 0.83$ ,  $P < 0.0001$ ), the mean %meth of combined *LINE-1* CpG sites was calculated for each subject and used in the association analyses.

### Statistical analysis

Non-normally distributed phenotypes were  $\log_{10}$  or negative inverse transformed. The general linear model (GLM) and the type III sum of squares (all subjects: sex included in the model; sex-specific: unadjusted) were used to compare the mean phenotype levels between subjects without (MetS-) and with (MetS+) MetS. Multiple linear regression analyses were performed to predict each MetS-related phenotype and MetS *per se* while including *LINE-1*%meth as an independent variable along with MetS-related potential confounding factors (see the Results section for more details about adjustments). After dividing the subjects into quartiles based on their *LINE-1*%meth and dichotomized by MetS- and MetS+ groups, a logistic regression analysis was performed to compute odds ratios (ORs) and Wald's confidence limits to evaluate whether *LINE-1*%meth quartiles can predict MetS risk. Finally, a multiple linear regression analysis was used to predict *LINE-1*%meth levels where age, sex and smoking were included in the model as independent variables. The statistically significant  $P$  value was set at 0.05. Statistical analyses were performed using SAS software V.9.2 (SAS Institute, Cary, NC, USA).

## Results

### Characteristics of the study subjects in MetS- and MetS+ groups

The characteristics of the subjects are presented in Table 1. No difference was observed in the mean age and smoking frequencies between MetS groups. Study subjects were severely obese with a mean body mass index of about 52 kg/m<sup>2</sup> and a mean age of about 35 years. Women in the MetS+ group had higher mean body mass index and waist circumference values compared to MetS- women. As expected, all the mean MetS-related phenotypes differed significantly between MetS groups.

### Relationship between *LINE-1*%meth levels, MetS and its related phenotypes

The mean *LINE-1*%meth in VAT of 186 severely obese subjects was of 75.8% (SD = 3.0%) with values ranging from 67.3 to 85.4%. Regression coefficients obtained specifically for *LINE-1*%meth in predictive models for each MetS-related phenotype and MetS *per se* are shown in Table 2. Negative associations were seen between *LINE-1*%meth levels and fasting glucose levels ( $\beta$  (95% confidence interval) = -0.04 (-0.08 to -0.01);  $P = 0.03$ ) and DBP (-0.65 (-1.26 to -0.05);  $P = 0.03$ ) when age, sex, waist circumference and smoking were included in the model. *LINE-1*%meth levels were also significantly predicting MetS *per se* (-0.04 (-0.06 to -0.01);  $P = 0.004$ ) when adjusted for age, sex and smoking. Of note, CpG site specific complementary analyses revealed that methylation levels of the first CpG site investigated in this assay were more closely related to the previous MetS phenotypes (fasting glucose:  $\beta = -0.04$ ,  $P = 0.007$ ; DBP:  $\beta = -0.52$ ,  $P = 0.04$ ; and MetS *per se*:  $\beta = -0.04$ ,  $P = 0.0002$ ), and also with the SBP ( $\beta = -0.88$ ,  $P = 0.01$ ), as compared to methylation levels of the second and the third CpG sites ( $P > 0.05$  for the whole model; data not shown).

### Prediction of MetS risk using quartiles of *LINE-1*%meth in VAT

The study subjects were divided into quartiles (Q) based on their *LINE-1*%meth levels (Q1: 67.3% to 73.8%; Q2: 73.8% to 75.0%; Q3: 75.0% to 77.3%; Q4: 77.3% to 85.4%) and dichotomized into MetS- and MetS+ groups. ORs were computed to evaluate whether some *LINE-1*%meth quartiles may be associated with greater risk of MetS, while taking the higher %meth quartile as the reference group. Greater risks were observed in the first (OR (95% confidence limits (CL); Q1: OR = 4.37 (1.59 to 12.01),  $P = 0.004$ ) and the second (Q2: OR = 4.76 (1.76 to 12.90),  $P = 0.002$ ) quartiles compared to Q4 (1.00), which was not the case for the third quartile (Q3: OR = 1.82 (0.70 to 4.71),  $P = 0.22$ ) when adjusting for age, sex and smoking (Table 3). While using a cut-off point of 75.0% for *LINE-1*%meth levels, the sensitivity and specificity of correctly categorizing subjects as having or not having MetS were 60.2% and 59.2%, respectively. Although the %meth cut-off point that would confer greater risk for MetS seems slightly different in additional site specific analyses, practically the same observation could be drawn, that is, quartiles with lower %meth levels for each CpG site were associated with greater MetS risk (Q1 for CpG<sub>1,2</sub>: OR = 2.82 to 8.34,  $P < 0.04$ ; Q2 for CpG<sub>1,2</sub>: OR = 2.80 to 8.16,  $P < 0.04$ ; Q3 for CpG<sub>1</sub>: OR = 2.71,  $P = 0.047$ ), as compared to the highest %meth quartile (Q4) (data not shown).

### Predictors of *LINE-1*%meth levels in VAT

The contribution of potential confounding factors in *LINE-1*%meth variation was tested using a multiple linear

**Table 1 Subject characteristics according to metabolic syndrome (MetS) status**

Phenotypes <sup>a</sup>	All subjects		Men		Premenopausal women	
	MetS-	MetS+	MetS-	MetS+	MetS-	MetS+
No. of subjects (n)	98	88	14	20	84	68
Smokers (n)	21 (21.4%)	22 (25.6%)	2 (14.3%)	4 (21.1%)	19 (22.6%)	18 (26.9%)
Age (years)	34.9 ± 8.1	35.3 ± 7.3	32.2 ± 8.9	36.8 ± 9.2	35.4 ± 7.9	34.9 ± 6.7
Body Mass Index (kg/m <sup>2</sup> ) <sup>b</sup>	49.8 ± 8.4	53.8 ± 10.8*	54.5 ± 8.8	56.8 ± 13.1	49.0 ± 8.1	52.9 ± 9.9**
Waist circumference (cm)	130.1 ± 17.0	139.4 ± 19.6**	151.6 ± 15.6	160.7 ± 20.7	126.8 ± 14.7	132.9 ± 14.0*
Fasting glucose (mmol/l) <sup>b</sup>	4.87 ± 0.39	5.68 ± 0.87***	5.06 ± 0.33	6.16 ± 0.75***	4.84 ± 0.39	5.53 ± 0.86***
Triglycerides (mmol/l) <sup>b</sup>	1.13 ± 0.37	2.20 ± 1.14***	1.08 ± 0.31	2.42 ± 1.41***	1.13 ± 0.38	2.13 ± 1.06***
High-density lipoprotein-cholesterol (mmol/l)	1.47 ± 0.26	1.09 ± 0.20***	1.27 ± 0.17	0.94 ± 0.15***	1.51 ± 0.38	1.13 ± 0.20***
Systolic blood pressure (mmHg)	130.2 ± 13.4	144.6 ± 17.2***	132.1 ± 12.8	150.2 ± 19.4**	129.9 ± 13.5	143.0 ± 16.4***
Diastolic blood pressure (mmHg) <sup>b</sup>	79.9 ± 9.3	90.4 ± 11.9***	78.8 ± 7.8	89.7 ± 13.2*	80.0 ± 8.7	90.6 ± 11.6***

<sup>a</sup>Data are shown as mean ± SD or n.

<sup>b</sup>Non-normally distributed phenotypes were transformed for the general linear model analysis: body mass index (negative inverse: -1/(X)), fasting glucose (-1/(X)), triglycerides (-1/(1 + X)) and diastolic blood pressure (-1/(X)).

\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.0001; for differences in mean phenotype levels between MetS- and MetS+ groups.

regression model including age, sex and smoking. The variance was partly accounted for by sex ( $\beta$  (95% confidence interval) = -3.21 (-4.27 to -2.15);  $P < 0.0001$ ; lower in women). Other factors were not significant (age: 0.04 (-0.02 to 0.09),  $P = 0.17$ ; smoking: 0.33 (-0.64 to 1.29),  $P = 0.50$ ).

## Discussion

This study revealed that *LINE-1*meth levels in VAT were associated negatively with fasting plasma glucose, blood pressure and MetS *per se* using the IDF definition [30]. Subjects situated in the quartiles with lower *LINE-1*meth levels had significantly greater risk to be affected by MetS when adjusted for age, sex and smoking. Sex was a significant predictor of *LINE-1*meth levels in VAT of the study subjects, which was not the case for age and smoking. To the best of our knowledge, this is the first study analyzing *LINE-1*meth levels in adipose tissue. Most of the published studies used peripheral blood cells [18,23,25,36-38],

tumorous vs non-tumorous tissues [39,40], and placental tissues [41] for *LINE-1* methylation analysis. *LINE-1* meth levels observed in VAT of the study subjects were comparable to levels reported in previous epidemiological studies using white blood cells (WBC) and the same methylation quantification method [18,23]. It thus suggests that *LINE-1*meth levels between VAT and WBC are comparable, even though the correlation between both compartments was not tested in the present study. Although further studies would be needed to investigate whether *LINE-1*meth levels in WBC are associated with MetS risk, as a more convenient biological sample for clinical and epidemiological purposes, this study underlines the presence of DNA methylation heterogeneity in the VAT of severe obese individuals. This finding supports the hypothesis of potential epigenetic changes in VAT, which may contribute to the development of metabolic perturbations in the presence of abdominal obesity. Since

**Table 2 Multiple linear regression analysis predicting metabolic syndrome (MetS)-related phenotypes while including *LINE-1* methylation level (*LINE-1*meth) in the predictive model**

Dependent variables	<i>LINE-1</i> meth		
	N	$\beta$ (95% CI) <sup>model</sup>	P value
Waist circumference (cm)	180	-0.51 (-1.33 to 0.32) <sup>A</sup>	0.23
Fasting glucose (mmol/l)	179	-0.04 (-0.08 to -0.01) <sup>B</sup>	0.03
Triglycerides (mmol/l)	179	0.01 (-0.04 to 0.06) <sup>B</sup>	0.64
High-density lipoprotein-cholesterol (mmol/l)	179	0.01 (-0.003 to 0.03) <sup>B</sup>	0.13
Systolic blood pressure (mmHg)	180	-0.71 (-1.55 to 0.13) <sup>B</sup>	0.10
Diastolic blood pressure (mmHg)	180	-0.65 (-1.26 to -0.05) <sup>B</sup>	0.03
MetS (n)	184	-0.04 (-0.06 to -0.01) <sup>A</sup>	0.004

Only coefficients ( $\beta$ ), 95% confidence intervals (95% CI) and P values accounted for by the independent variable *LINE-1*meth levels are shown in the table. The coefficient  $\beta$  corresponds to the change in the dependent variable per unit change in *LINE-1*meth, given that all the other variables in the model remain constant.

Model A: *LINE-1*meth, age, sex and smoking.

Model B: *LINE-1*meth, age, sex, smoking and waist circumference.

**Table 3 Logistic regression analysis evaluating the association between *LINE-1* methylation level (*LINE-1*%meth) quartiles and metabolic syndrome (MetS) risk**

<i>LINE-1</i> %meth quartiles	MetS-, <i>n</i> = 98	MetS+, <i>n</i> = 86	Wald's $\chi^2 = 12.3$ , <i>P</i> = 0.007 <sup>a</sup> , OR (95% CL)	<i>P</i> value
Q1: 67.3% to 73.8%	21 (0.22)	25 (0.29)	4.37 (1.59 to 12.02)	0.004
Q2: 73.8% to 75.0%	19 (0.19)	27 (0.31)	4.76 (1.76 to 12.90)	0.002
Q3: 75.0% to 77.3%	28 (0.29)	18 (0.21)	1.82 (0.70 to 4.71)	0.22
Q4: 77.3% to 85.0%	30 (0.31)	16 (0.18)	1.00	-

Odds ratios and *P* values computed by the logistic regression analysis were obtained while including potential confounding factors in the model, namely age, sex and smoking. The fourth *LINE-1*%meth quartile (Q4) was set as the reference group.

<sup>a</sup>Corresponds to the Wald's  $\chi^2$  and *P* value for the effect of *LINE-1*%meth quartiles. For the whole model, Wald's  $\chi^2 = 14.0$  and *P* value = 0.03.

CL = Wald's confidence limits.

differential gene expression patterns were previously observed in VAT of obese with versus without MetS [7], gene-specific DNA methylation analysis in VAT are warranted to better understand the contribution of epigenetics in VAT function and, consequently, in obesity-related metabolic disorders.

The study results showed higher plasma glucose and DBP levels, along with greater risk for MetS, in presence of lower *LINE-1*%meth levels in VAT. However, this relationship is in the opposite direction of what Pearce *et al.* reported [24]. In their samples of 228 Caucasian individuals aged 49 to 51 years from the Newcastle Thousand Families Study (Newcastle upon Tyne, UK), they found a positive association between peripheral blood *LINE-1*%meth and fasting glucose levels. They also observed positive and negative associations, respectively, with plasma triglyceride and HDL-cholesterol levels after adjustment for sex [24]. In contrast, Kim *et al.* reported no significant association between blood *LINE-1*%meth levels and fasting glucose, triglyceride, HDL-cholesterol, SBP and DBP in healthy male and female Koreans [42]. Since their study sample was relatively small (*n* = 86) and that no adjustments for sex were performed, it is difficult to clearly delineate the direction of the relationship between *LINE-1*%meth and MetS-related phenotypes. However, since most studies reported that lower *LINE-1*%meth levels in peripheral blood cells were associated with lower HDL-cholesterol levels [25] and with higher risk of incident ischemic heart disease and stroke [23], it is tempting to suggest that lower *LINE-1*%meth levels may be associated with MetS and its related phenotypes.

Regarding potential predictors of *LINE-1*%meth, lower methylation levels were observed in VAT of women as compared to men in this study, which was also reported in several studies using WBC for *LINE-1*%meth quantification [19,38,43,44]. The reason for this sex difference still remains to be clarified, but it does not appear to be related to male/female hormone differences [44,45]. While there is growing evidence that WBC global DNA methylation reduces with aging, several studies did not observe an association between *LINE-1*%meth levels with either age [38,44], or active cigarette smoking in

adults [38]. Consistent with these previous studies, age and smoking were not significant predictors of *LINE-1*%meth levels in VAT of the present study subjects.

As reported in a previous study, many genes were differentially expressed in VAT of MetS- versus MetS + severely obese men [7] and epigenetic mechanisms have been postulated as potentially mediating differential expressions [33]. There is recent evidence supporting a correlation between *LINE-1* and gene-specific %meth levels, which would depend upon the genomic region and type of tissue analyzed [46]. However, the nature of this relationship still remains underexplored. The relevance of *LINE-1* methylation quantification as a marker of global DNA methylation is supported by the fact that *LINE-1* is the most prevalent repetitive sequence in the human genome (approximately 17% of the genome) [20] and that about one-third of DNA methylation in the genome occurs in these elements [35]. Some reports have also shown that *LINE-1*%meth levels significantly correlated with other methods assessing global DNA methylation, such as genomic 5-methyl cytosine content [47] and luminometric methylation assay (LUMA) [46]. Bisulfite sequencing of repetitive elements, as performed here for *LINE-1* elements, is a quicker and easier method to assess global DNA methylation as compared to genomic 5-methyl cytosine quantification [35]. Thus, all the above observations would support the utility of assessing *LINE-1*%meth as a marker of global DNA methylation in VAT of our study subjects, and suggest that the variability observed in *LINE-1*%meth levels may be suggestive of concomitant variability in gene-specific %meth levels potentially associated with differential gene expression and MetS phenotypes. It should be mentioned that methylation quantification of other repetitive elements, such as Alu repeats, is sometimes performed to assess global DNA methylation [18], which was not measured in this study. Correlations between *LINE-1* and Alu methylation levels were observed in some but not all studies [18,48] and may depend on the type of tissues examined and the responses to cellular stressors and environmental exposures [18]. *LINE-1* and Alu repeats represent distinct measures of dispersed DNA

methylation [19] and it is unknown whether the same associations observed in this study would be seen with Alu methylation levels. *LINE-1* methylation assessment was chosen in this study over Alu because of previous associations between *LINE-1* methylation levels and MetS phenotypes [24,25], as well as with ischemic heart disease and stroke [23]. Another reason for investigating *LINE-1* elements is its function. There are approximately 3,000 to 5,000 full length (approximately 6 kb) *LINE-1* sequences throughout the genome, which comprise the 5' region targeted in the *LINE-1*meth analysis, in which approximately 60 to 100 elements are still capable of retrotransposition [49]. It has been postulated in cancer that demethylation of *LINE-1* elements may increase their retrotransposable activity, induce genomic instability, and deregulate transcriptional activity of specific genes [50]. Whether such a portrait would also be seen in other common complex diseases is unknown.

Some study limitations need to be outlined, such as the possibility of reverse causality [17]. It is indeed possible that the development of obesity-related metabolic complications may have induced changes in *LINE-1*meth rather than the reverse. Furthermore, DNA methylation of *LINE-1* elements has been associated with several environmental exposures, such as air pollution, metal exposures, persistent organic pollutants, as well as alcohol and dietary folate consumption (reviewed in [38]). Whether these confounding factors have influenced *LINE-1*meth variability in this study and consequently changed the relationship seen with MetS is unknown. It may however need some concerns since several persistent organic pollutants may accumulate in adipose tissue, which have been attributed with obesity development, type 2 diabetes and related metabolic impairments in human and animal models [51,52]. Also, VAT is a heterogeneous tissue composed of different cell types, including infiltrated lymphocytes [53], which are increased in presence of obesity and associated with insulin resistance [54]. It has been shown that the percentage of lymphocytes in blood cell counts was negatively associated with blood *LINE-1*meth levels [18]. Whether our MetS+ subjects have greater lymphocyte infiltration in their VAT, which may influence *LINE-1*meth values obtained in this study, is a possibility which warrants further investigation. Finally, Zhang *et al.* have previously reported that some variability in *LINE-1* methylation levels exist in peripheral leukocytes among cancer-free individuals of different ethnicities [43]. These authors proposed that genetic polymorphisms in the folate metabolism, which provides methyl groups for DNA methylation [55], or other unknown genetic and environmental factors, may contribute to ethnic differences in global DNA methylation [43]. It thus suggests that associations observed among Caucasians in the present study may not necessarily be generalized to all ethnic groups and may potentially be influenced by genetic polymorphisms.

## Conclusions

In summary, this study revealed that lower *LINE-1*meth levels in VAT of non-diabetic severely obese subjects was associated with higher plasma glucose and DBP levels, and with a greater risk to be affected by MetS. This investigation also point out to the necessity to pursue gene-specific methylation analysis among differentially expressed genes in the VAT of severely obese subjects MetS- and MetS+ [7] to better understand the involvement of epigenetic regulation in the development of obesity-related metabolic complications.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

VT initiated the study, performed the statistical analyses, interpreted the data and drafted the manuscript. AT, YD, and LP participated to the elaboration of the study design. AB was in charge of the methylation analysis. SM, SB, OL, LB sampled blood and adipose tissue from the study subjects. MCV conceived and designed the study. All authors read and approved the final manuscript.

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