JOURNAL OF THE AMERICAN COLLEGE OF NUTRITION
https://doi.org/10.1080/07315724.2018.1553116

LINE-1 in Obesity and Cardiometabolic Diseases: A Systematic Review

Lílian L. Lopes, Josefina Bressan, Maria do Carmo G. Peluzio, and Helen Hermana M. Hermsdorff

Department of Nutrition and Health, Universidade Federal de Viçosa (UFV), Viçosa, Minas Gerais, Brazil

ABSTRACT

Epigenetic mechanisms may play an important role in the etiology of obesity and cardiometabolic diseases, by activating or silencing the related-genes. Scientific evidence has suggested that *LINE-1* methylation is associated with body composition and obesity-related diseases, including insulin resistance, type 2 diabetes mellitus, and cardiovascular disease (CVD). It also has been evaluated as predictor of weight loss. The studies' results are still conflicting, and positive and negative associations have been found to *LINE-1* methylation regarding adiposity and cardiometabolic markers. Overall, this review presents observational (cross-sectional and longitudinal) studies and interventions (diet, exercises, and bariatric surgery) that evaluated the relationship of the *LINE*-1 methylation with obesity, weight loss, dyslipidemias, hypertension, insulin resistance, CVD, and metabolic syndrome.

TEACHING POINTS

- Epigenetic mechanisms may play an important role in the etiology of obesity and cardiometabolic diseases.
- Many studies have related methylation of *LINE-1* with cardiometabolic diseases; however, the results are still controversial.
- The relationship between the etiology of chronic diseases and the methylation of *LINE-1* is not fully elucidated.
- With advances in epigenetic studies, related mechanisms may be early biomarkers in weight change and cardiometabolic risk.

ARTICLE HISTORY
Received 7 August 2018
Accepted 24 November 2018

KEYWORDS
Epigenetic; DNA methylation; overweight; cardiovascular disease; metabolic syndrome

Introduction

Epigenetic modifications, contrary to genetic modifications that lead to change in DNA sequence, are heritable alterations without variation in DNA sequence, which may change according to cellular age, development, and differentiation (1, 2). In this context, epigenetic mechanisms may play an important role in the etiology of chronic diseases, such as obesity or metabolic syndrome (MS), by activating or silencing genes involved in these diseases (1).

DNA methylation is the most investigated epigenetic mechanism in obesity and MS (3–5). Although methylation of specific genes involved in energy metabolism, food intake, lipid metabolism, and inflammation has been associated with increased adiposity and insulin resistance (5–9), weight loss could affect the methylation pattern of these genes (3).

Global DNA methylation can be assessed through repetitive genome elements such as *LINE-1* (*long interspersed nucleotide element-1*), associated with genomic instability and chromosomal abnormalities in the gene promoter regions, which can be activated or silenced according to the pattern of methylation. *LINE-1* is the most repeated sequence in the genome, corresponding to about 17% to 21% of human DNA (10–12). In addiction, one-third of genome methylation occurs in these elements, which justifies its use as a global marker (10, 13).

Recent scientific evidence suggests that changes in *LINE-1* methylation are associated with prominent obesity-related diseases, including cancer (14, 15), type 2 diabetes mellitus (T2DM) (16), and cardiovascular disease (CVD) (17, 18). However, the results are controversial (10, 19–21), and the mechanisms involved have not yet been established.

Therefore, the aim of this review is to present studies on the evaluation of *LINE-1* methylation and its relationship with obesity and other cardiometabolic diseases, such as dyslipidemias, CVD, and MS.

Methodology

For this review, we searched in the PubMed, MEDLINE (EBSCO), SCOPUS, Scielo, and Web of Science databases, without date or language as limitation. The search terms used were: *LINE1, LINE-1, long interspersed elements, L1Hs, repetitive elements, Long Interspersed Nucleotide Elements, L1 Elements, LINE-1 Elements, obesity, cardiovascular disease, dyslipidemia,* and *metabolic syndrome*. We used the association between these terms and expressions with the Boolean connectors (AND, OR, and NOT).The search was performed for title, subject, and summary.

CONTACT Helen H. M. Hermsdorff helenhermana@ufv.br Department of Nutrition and Health, Universidade Federal de Viçosa, Av. P.H. Rolfs s/n, Campus Universitário, 36570-900, Viçosa, Minas Gerais, Brazil.

The titles and abstracts of all studies identified by the search were selected according to inclusion criteria: original papers including assessment of the *LINE-1* methylation and association analyses with the occurrence and/or risk of overweight, obesity, dyslipidemia, CVD, and MS or with their recognized markers (e.g., body mass index [BMI], high-density lipoprotein cholesterol [HDL], blood pressure, and glucose).

Potentially relevant articles were read in full for evaluation according to inclusion criteria. We excluded all articles with animal models and in vitro studies, studies having populations with cancer, hormonal, or hepatic problems, among other diseases, populations exposed to environmental pollutants, and those that did not analyze *LINE-1* or did not study selected cardiometabolic disease or their related markers as outcomes. In addition, editorials, articles with

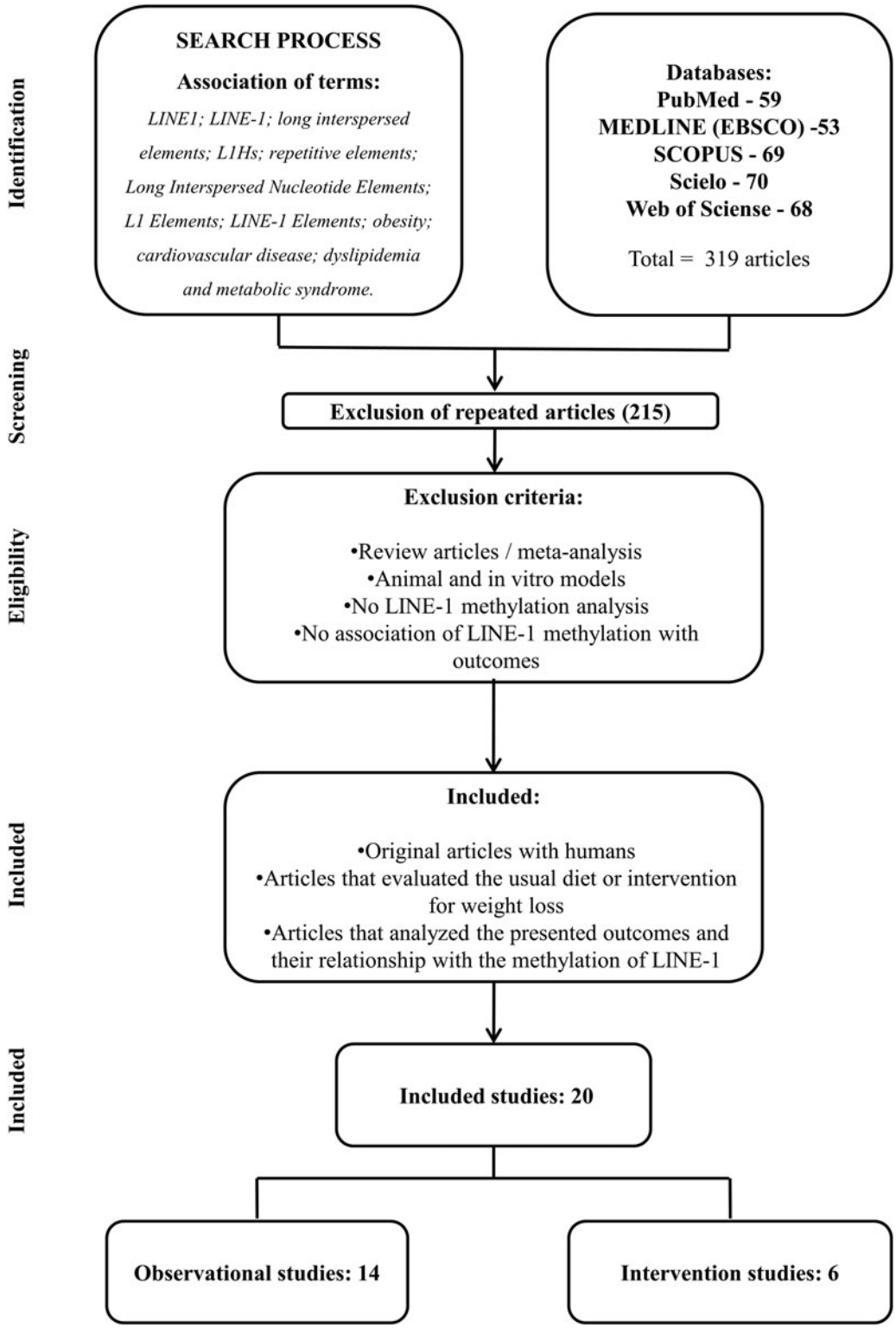

Figure 1. Flowchart of selection of studies.

Table 1. Association of *LINE-1* Methylation With Indicators of Adiposity and Cardiometabolic Markers: Observational Studies (2007–2017).

Authors and year of publication	Type/duration of study	Subjects	Biological sample for analysis of *LINE-1*	Main results
Geisel et al., 2007 (22)	Cross-sectional	48 subjects (M and W) Group 1: 22 with ESRD Group 2: 26 controls (healthy) Age: 68 ± 10.9 (ESRD) and 63 ± 11.4 (controls) years BMI: NA	PBMC	*LINE-1* methylation was higher in the ESRD group.
Baccarelli et al., 2010 (17)	Longitudinal (7 years)	742 subjects (M) Age: 73.8 (55.3–92.6) years BMI: 28.1 (19.1–52.6) kg/m^2	White cells	Hypomethylation of *LINE-1* was associated with higher concentrations of VCAM-1 and was more prevalent in subjects without ischemic heart disease or stroke.
Cash et al., 2011 (18)	Cross-sectional	355 subjects (88 M and 267 W) Group 1: residents of American Samoa Group 2: residents of Samoa Age: Group 1 (31.6 y ± 7.1) and Group 2 (31.2 ± 7.3) years BMI: Group 1 (M: 34.8 ± 6.6 and W: 36 ± 9.2) and Group 2 (M: 28.7 ± 5.4 and W: 31.1 ± 5.8) kg/m^2	White cells	Men had higher methylation of *LINE-1* than women. Lower levels of *LINE-1* methylation were associated with higher concentrations of LDL and lower levels of fasting HDL.
Michels et al., 2011 (23)	Cross-sectional	319 mother–infant dyads Age: 32 (18–45) years BMI: NA	Umbilical cord and placenta cells	Newborns with low or high birth weight had higher *LINE-1* methylation compared to normal weight infants.
Turcot et al., 2012 (24)	Cross-sectional	186 subjects (34 M and152 W) Group 1: Without MS (14 M and 84 W) Group 2: With MS (20 M and 68 W) Age: Group 1 (34.9 ± 8.1), Group 2 (35.3 ± 7.3) years BMI: Group 1 (49.8 ± 8.4), Group 2 (53.8 ± 10.8) kg/m^2	Visceral adipose tissue cells	*LINE-1* methylation was negatively associated with fasting glycemia, diastolic blood pressure and MS. *LINE-1* hypomethylation was most strongly associated with the increased risk of MS in the presence of obesity.
Alexeeff et al., 2013 (25)	Longitudinal (10 years)	789 elderly (M and W) Age: 74 ± 6.7 years BMI: 28.1 ± 4.1 kg/m^2	White cells	No association between the *LINE-1* methylation over time in blood pressure
Perng et al., 2013 (26)	Longitudinal (30 months)	553 children (45.9% boys) Age: 5 to 12 years (8.86 ± 1.7 years)18.2% overweight/obese	White cells	The lower *LINE-1* methylation was related to the development of adiposity, only in boys.
Piyathilake et al., 2013 (21)	Cross-sectional	470 child-bearing age women (with abnormal cervical cells) BMI: 28.6 ± 8.6 kg/m^2	PBMC	*LINE-1* hypomethylation was associated with excess body weight and higher HOMA-IR, especially in the presence of lower folate plasma concentrations.
Perng et al., 2014 (19)	Cross-sectional	987 subjects (457 M and 504 W) Age: 61,4 ± 9,9 years BMI: 220 subjects (<25 kg/m^2), 382 (25–29 kg/m^2), 234 (30–34.9 kg/m^2) and 46 (≥ 40 kg/m^2)	White cells	BMI and plasma homocysteine concentrations were positively associated with *LINE-1* methylation. Participants with BMI > 40 kg/m^2 presented higher *LINE-1* methylation compared to those with normal BMI.
Guarrera et al., 2015 (27)	Longitudinal (4 to 5 years)	584 subjects (M and W, EPICOR and EPIC-NL cohorts) Group 1: 292 MI cases Group2: 292 controls	White cells	Group 1 presented *LINE-1* hypomethylation in relation to Group 2. When stratified by gender, only males maintained the pattern of hypomethylation.
Manzardo and Butler, 2016 (28)	Cross-sectional	91 adults (46 M and 45 W) Age: 35.1 ± 9.2 years BMI: Group 1, 25 normal weight (23.2 ± 0), Group 2, 26 obese (42.6 ± 15.4), Group 3, 39 PWS (34.4 ± 9.1)	White cells	There were no differences between groups regarding *LINE-1* methylation.
Marques-Rocha et al., 2016 (29)	Cross-sectional	156 subjects (91 Wand 65 M) Age: 23.1 ± 3.5 years BMI: 22 ± 2.9 kg/m^2	White cells	Adiposity was lower as well as fat-free mass was higher among subjects with higher *LINE-1* methylation. Individuals with higher methylation *LINE-1* had higher daily intake of calories, iron, and riboflavin.

(*continued*)

Table 1. Continued.

Authors and year of publication	Type/duration of study	Subjects	Biological sample for analysis of *LINE-1*	Main results
Carraro et al., 2016 (10)	Cross-sectional	40 (9 M and 31 W) Age: 28.9 ± 7.0 (22-53) years BMI: 22.4 ± 3.4 kg/m^2	PBMC	*LINE-1* hypermethylation was positively associated with markers of adiposity (BMI and WC), insulin resistance, and lower quality of diet.
Dunstan et al., 2017 (20)	Cross-sectional	431 adolescents (M and W) Age: 12.9 ± 1.7 years BMI: 65.67% (<85th percentile), 16.36 % (85th to 95th percentile) and17.97% (≥95th percentile)	Saliva cells	There were no associations of methylation of *LINE-1* with obesity related-markers (BMI, % body fat, and waist circumference).

Note. AI: acute infarction; BMI: body mass index; BP: blood pressure; EPIC-NL: Dutch EPIC cohort; EPICOR study: European Prospective Investigation into Cancer and Nutrition cohort; ESRD: end-stage renal disease; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; M: men; MI: myocardial infarction; NA: not shown/not applicable; PBMC: peripheral blood mononuclear cells; PWS: Prader–Willi syndrome; MS: metabolic syndrome; T2DM: type 2 diabetes mellitus; VCAM: vascular cell adhesion molecule-1; W: women; WC: waist circumference.

Table 2. Effect of *LINE-1* Methylation in Weight Loss: Intervention Studies (2013–2016).

Authors and year of publication	Type/duration of study	Subjects	Biological sample for analysis of *LINE-1*	Main results
Remely et al., 2013 (30)	Controlled intervention: GLP-1 agonists for T2DM and nutritional counseling according to OEGE. 4 months	56 adults (M and W) Group 1: 24 subjects with T2DM (58.36 ± 9.35 years) Group 2: 14 obese (39.63 ± 15.18 years) Group 3: 18 controls (normal weight) (25.67 ± 3.09 years)	White cells	There were no differences between the groups and over time for the methylation of *LINE-1*.
Duggan et al., 2014 (31)	Randomized and controlled intervention independent and combined effects of reduced-calorie weight-loss diet, and exercise program, vs. control. 12 months	300 W Age: 50–70 years Group 1: 118 (caloric restricted diet) Group 2: 117 (moderate to vigorous exercise) Group 3: 117 (diet and exercise) Group 4: 87 (control/without intervention)	White cells	There was no difference in the *LINE-1* methylation in any intervention or control group. Weight loss was not associated with *LINE-1* methylation at 12 months.
Martín-Núñez et al., 2014 (1)	Prospective cohort intervention: Program with regular controls to achieve goals for dietary habits, exercise, and weight within the Mediterranean dietary pattern. 1 year	310 subjects (M and W) Age: 45 to 65 years Group 1: 155 (change in glycemia or T2DM), exercise and Mediterranean diet Group 2: 155 (control), general recommendations on diet and physical activity Intervention kg/m^2 (start 31.04 ± 5.1 and end: 30.2 ± 5.12 kg/m^2)	White cells	Individuals with lower adherence to the Mediterranean diet had greater changes in *LINE-1* methylation. DNA methylation levels were associated with weight change and adherence to a Mediterranean diet.
Garcia-Lacarte et al., 2015 (32)	Controlled intervention The control diet (based on the AHA) and the RESMENA diet (diet for MS reduction) 8 weeks.	96 subjects with MS (M and W) Age: 48 (45.4–50.6) (Low responders) and 51 (48–54) (high responders) years Group 1: RESMENA diet (40% CARB, 30% PTNA and 30% LIP) Group 2: AHA diet (55% CARB, 15% PTN and 30% LIP)	PBMC	*LINE-1* methylation was higher (5.4%) in high responders (>8% weight loss) for calorie restriction treatment.
Nicoletti et al., 2015 (33)	Controlled intervention: The participants followed an energy-restricted dietary program, and bariatric surgery in Group 3. 6 months	45 M Group 1: control ($n = 9$), normal weight Group 2: energy restriction ($n = 22$), obese Group 3: bariatric surgery ($n = 14$), obese subjects undergoing diet and surgery Age: Group 1 (31.7 ± 8.6), Group 2 (52.6 ± 9.9), and Group 3 (35.5 ± 10.1) years	White cells	*LINE-1* methylation did not change after weight loss
Martín-Núñez et al., 2016 (34)	Surgery intervention: different bariatric surgery procedures. 6 months	60 T2DM 60 subjects (H and M) Group 1: Obese nondiabetic who underwent RYGB (44.2 ± 8.02 years) Group 2: Obese diabetic who underwent RYGB (41.5 ± 9.5 years) Group 3: Obese nondiabetic who underwent LSG (42.5 ± 9.2 years) Group 4: Obese diabetic who underwent LSG (51.7 ± 7.7 years)	White cells	There were no differences in *LINE-1* methylation over time, in relation to the groups (diabetic versus nondiabetic) and according to the bariatric surgery procedure. *LINE-1* methylation was positively associated with body weight at baseline.

Note. AHA: American Heart Association diet; BMI: body mass index; BP: blood pressure; CARB: carbohydrates;T2DM: type 2 diabetes mellitus; LIP: lipids; LSG: laparoscopic sleeve gastrectomy; M: men; MI: myocardial infarction; MS: metabolic syndrome; OEGE: Austrian Nutrition Society; PBMC: peripheral blood mononuclear cells; PTN: protein; RYGB: Roux-en-Y gastric bypass; W: women.

insufficient data, summaries of meeting presentations, reviews, and meta-analyses were excluded.

However, other articles were included in the present review in order to improve the discussion.

The Figure 1 represents the search and selection process.

Results and discussion

In accordance to research methodology, we obtained 21 articles, which are described in Table 1 (Observational Studies) and Table 2 (Intervention Studies).

LINE-1 methylation in observational studies

This review identified 14 observational studies that investigated different population groups, mostly adults and the elderly, who were overweight, obese, with MS, acute myocardial infarction, end-stage renal disease (ESRD), or Prader–Willi syndrome, and evaluated indicators of adiposity (weight, BMI, body fat and waist circumference), energyintake, blood pressure, carbohydrate metabolism markers (fasting glycemia and HOMA index), low-density lipoprotein cholesterol (LDL) and HDL, homocysteine, and plasma folate, as well as *LINE-1* methylation.

A majority of the studies ($n = 9$) assessed the association between *LINE-1* methylation and an indicator of adiposity (weight, BMI, body fat, and waist circumference) or the presence of overweight/obesity. Three studies found a positive relationship, suggesting that *LINE-1* methylation could be an epigenetic mechanism by which obesity modulated inflammation and cardiometabolic risk factors. However, four studies found a negative relationship and two studies found no significant relationship.

In fact, environmental stimuli in the early stages of life may induce long-lasting changes in DNA methylation profiles related to obesity and cardiometabolic disease (26). Thus, it is important to conduct more studies on children, as well as with possible obesity markers and cardiometabolic diseases associated with epigenetic markers.

Some authors analyzed the association between methylation levels and insulin resistance; however, the results were divergent. While Carraro et al. (10) found a positive association between the methylation of *LINE-1* and HOMA-IR index, Piyathilake et al. (21), on the other hand, found an association between hypomethylation and increased HOMA-IR and weight, especially in the presence of low folate concentrations (10, 21).

Among the risk factors for CVD, changes in blood lipids have been widely discussed with respect to the genesis of these diseases. Cash et al. (18) evaluated the association between *LINE-1* methylation and factors associated with metabolic and cardiovascular diseases. They observed that men had higher methylation levels compared to women, where lower *LINE-1* methylation was associated with higher fasting LDL levels in women and lower levels of fasting HDL. A possible mechanism for the DNA hypomethylation observed in cardiovascular disease is an influx of inflammatory cells in the blood triggered by inflammation, resulting in a change in blood cell profile and consequent alterations in DNA methylation levels (18).

Corroborating with this result, Baccarelli et al. (17) evaluated whether *LINE-1* methylation was associated with VCAM-1, inter-cellular adhesion molecule-1 (ICAM-1), and C-reactive protein (CRP), and found that, *LINE-1* hypomethylation was associated with increased circulating levels of vascular cell adhesion molecule-1 (VCAM-1), particularly in subjects without CVD. VCAM-1 has been described as a predictor mechanically related to cardiovascular disease because it is rapidly expressed in pro-atherosclerotic conditions and has been shown to play a critical biological role in several stages of atherosclerosis (17).

Homocysteine is another recognized marker of CVD, present in increased concentrations in individuals at cardiovascular risk. In this regard, Geisel et al. (22) found an association between plasma homocysteine and *LINE-1* methylation in patients with ESRD; however, no association was found between homocysteine and *S*-adenosylhomocysteine (SAH), which can be explained by the fact that the physiological elimination pathway of SAH in urine is altered in ESRD patients (22). On the other hand, Perng et al. (19) found that higher plasma concentrations of homocysteine and a higher BMI were related to higher *LINE-1* methylation. With respect to homocysteine, this result was unexpected because, under ideal conditions, a deficiency in methyl donor micronutrient leads to an increase in homocysteine and a decrease in DNA methylation (19).

In this context, the authors present some hypotheses to explain these conflicting results: DNA methylation can also be modulated by other alternative mechanisms related to carbon 1 metabolism, such as systemic inflammation (10, 19). Another hypothesis is that the concentrations of folate, a nutrient involved in the methylation process, could be a limiting factor, where low concentrations of this nutrient can lower the level of methylation and are related to overweight (21). Thus, habitual intake of foods fortified with methyl donors (such as folate) may interfere with the results, showing no association between the variables studied (35). Also, a possible explanation for these conflicting results is that epigenetic changes, such as DNA methylation, are cell dependent, and among the studies there was no standardization of the type of tissue used, nor did the authors specify the amount of cells used. DNA methylation occurs primarily in the CpG islands and can affect gene expression by modifying the degree to which DNA is accessible to promoters or suppressors (20). Considering that many of the mechanisms of methylation are unknown, there may be errors associated with the measurements, and in addition, there is a gap in the evaluation of temporal changes in weight and epigenetic changes (35). Altogether, these findings demonstrate the need for further research, particularly prospective studies, in order to elucidate the relationship between *LINE-1* methylation, adiposity indicators, and cardiometabolic risk factors.

LINE-1 *methylation in intervention studies*

We selected six studies that evaluated *LINE-1* methylation before and after the follow-up of nutritional intervention,

nutritional counseling, physical activity, and/or bariatric surgery—all aimed at weight loss and metabolic improvement. The volunteers were men and women, overweight and/or with T2DM and SM. The overall objective of the studies was to assess whether there is any association between DNA methylation levels and weight and if methylation levels change after nutritional intervention. The studies presented diverging results.

According to the studies, the intervention period (6 to 12 months) was sufficient to observe differences in weight loss and metabolic improvement, but insufficient to detect changes in global DNA methylation, at least using *LINE-1* as a marker. Thus, the absence of associations between weight loss and methylation of *LINE-1* may be due to the insufficient time of exposure to the treatment.

However, some authors did not present information on the health and/or nutritional status of the volunteers prior to the study, as to whether they were previously following a specific diet or presented significant weight gain or loss before the intervention studies, which may interfere with DNA methylation.

In fact, there is no consensus in the literature on the short-term effect that lifestyle changes have on epigenetic changes. Studies have shown changes mainly in specific genes, but not in relation to global DNA methylation (*LINE-1*). Thus, further studies are needed to determine whether *LINE-1* is a stable epigenetic marker or, conversely, whether it is susceptible to modification by external factors such as changes in lifestyle or surgical intervention (34). In addition, most of the studies analyzed *LINE-1* methylation in the leukocytes and this is discussed in relation to the conflicting results. DNA methylation measured in these cells has been recognized as a good marker, replacing less accessible tissues that are directly involved in the disease, with interesting results for methylation of specific genes (10, 29, 36–39).

However, the authors of the studies in this review discussed their results carefully, due to several factors that may affect the pattern of DNA methylation, and, since few nutritional intervention studies with humans evaluate the methylation of *LINE-1* and the results are inconsistent. In addition, adipose tissue during weight loss appears to exhibit tissue-specific methylation changes that are not reflected in peripheral blood mononuclear cells (PBMC) DNA (31).

Conclusions

Despite the promising perspective on the application of epigenetic factors as early biomarkers in weight change and cardiometabolic risk, the number of studies is still insufficient to establish the direction of this relationship. Similarly, weight loss intervention studies have not been able to establish variation in the methylation pattern of *LINE-1*, as already established in DNA methylation studies, using microarray or determination of specific gene pattern.

In this sense, the differences in methodology observed in the studies included in the review provides an additional source of variability, reinforcing the need for more studies aimed at determining the cause-effect relationship between weight loss or gain, *LINE-1* methylation, and cardiometabolic risk.

Acknowledgments

We thank the CAPES Foundation (Ministry of Education, Brazil, Financial Code 001), the Foundation for Research Support of the State of Minas Gerais (FAPEMIG, State of Minas Gerais, Brazil), and National Council for Scientific and Technological Development (CNPq, Ministry of Science and Technology, Brazil) for financial support for related projects. J .Bressan, M. C. G. Peluzio, and H. H. M. Hermsdorff are CNPq fellows.

Conflicts of interest

The authors declare that they have no conflict of interest.

Funding

The work was support by the National Council for Scientific and Technological Development – CNPq (Ministry of Science and Technology, Brazil, process 308772/2017-2), the CAPES Foundation (Ministry of Education, Brazil, Finance Code 001) and the Foundation for Research Support of the State of Minas Gerais – FAPEMIG (State of Minas Gerais, Brazil, CDS-APQ-02425-15).

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