Methylation levels of LEP and POMC genes after intervention with dietary folate and hazelnut oil in the lipid profile of overweight women

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Abstract

Background

Obesity contributes to several cardiometabolic diseases, such as dyslipidemia, as a result of an unhealthy lifestyle and genetic factors. The methylation profile of genes involved in appetite control and metabolism, such as *LEP* (leptin) and *POMC* (proopiomelanocortin) and altered lipid levels can contribute to obesity, and these epigenetic changes have been associated with the effects of diet composition. The objective of this study was to evaluate the methylation levels of *LEP* and *POMC* genes and lipid profile values after intervention with dietary folate and hazelnut oil in overweight women.

Methods

Double-blind, placebo, controlled intervention study with 40 overweight adult women. Participants were randomized into four groups for 8 weeks: G1, 300 g of vegetables and 191 µg / day of folate and hazelnut oil; G2, 300 g of vegetables and 191 µg / day of folate and placebo; G3, 300 g of vegetables and 94 µg / day of folate and hazelnut oil; G4, the individuals were only accompanied. In addition to the levels of methylation, food consumption, anthropometric measurements, biochemical variables of lipid profile were evaluated.

Results

After the intervention, the participants presented reduction in the methylation levels of the studied genes in the three intervention groups in the *LEP* gene: G1 ($p = 0.00$), G2 ($p = 0.00$) and G3 ($p = 0.00$); in the *POMC* gene: G1 ($p = 0.01$), G2 ($p = 0.02$) and G3 ($p = 0.01$), and in the lipid profile, G1 reduced the levels of LDL-c ($p = 0.04$), HDL-c ($p = 0.00$) and Triglycerides ($p = 0.04$); in G3 there was a reduction in total cholesterol levels ($p = 0.00$), LDL-c ($p = 0.00$) and HDL-c ($p = 0.00$), and in G4 there was a reduction in total cholesterol values ($p = 0.00$), LDL-c ($p = 0.00$), HDL-c ($p = 0.00$) and triglycerides ($p = 0.00$), and also, an association in G2 between the *POMC* methylation levels with triglycerides ($p = 0.00$).

Conclusion

The study provided evidence of a normocaloric intervention with dietary folate and hazelnut oil supplementation on the methylation levels of *LEP* and *POMC* genes and the role they can play in lipid metabolism.

Trial registration

ISRCTN, NCT04523532. Registered 21 August 2020, <https://clinicaltrials.gov/ct2/show/NCT04523532>

Introduction

The prevalence of obesity is increasing globally, not only in well developed countries, but also in developing countries, it has an impact on public health worldwide and contributes to several cardio-metabolic diseases such as dyslipidemia [1].

Altered blood lipid levels contribute to the pathophysiology of complex diseases, including diabetes and cardiovascular diseases (CVDs) - two of the leading causes of morbidity and mortality in industrialized and developing countries [2-3].

Dyslipidemias are mainly the result of a harmful lifestyle: poor nutrition, sedentariness, overweight and genetic factors [4-5]. Lipid values may be affected by altered levels of gene expression. These levels, however, are also regulated by the methylation of DNA, which is one of the most studied mechanisms in the field of epigenetics and can therefore affect lipid levels [6].

DNA methylation is dynamic over time and responsive to the environment; therefore, this mechanism can also change the response to blood lipid levels [7]. Available Epigenome Wide Association Studies (EWAS) offer the possibility to identify associations between DNA methylation in new gene loci involved with serum lipid levels [8].

Several dietary fats have been associated with changes in DNA methylation, such as polyunsaturated fats (PUFAs - omega 6) and TNF- α gene (HERMSDORFF et al., 2013), components in hazelnut oil and *ADRB3* gene [9], other fatty acids and *CLOCK* [10], triglycerides and genes *ABCG1*, *ABCA1*, *PHGDH*, *TOMM20* [11-14]. The association between the DNA methylation profile and the transcription of several genes involved in lipidic metabolism with blood lipid levels, was reported by RODHE et al., [15].

These epigenetic changes have been associated with the effects of diet composition on health and disease or the long-term effects of gene-environment interactions [16-17]. In this sense, among the diet composition, folate is a nutrient known to participate in the process of DNA methylation and nucleotide reactions of biosynthesis. It is involved in the formation of radical S-adenosylmethionine (SAM), which serves as a methyl donor for the methylation of DNA.

There is growing evidence that the DNA methylation profile can contribute to obesity. In fact, studies on the methylation of candidate genes in animal and human models have demonstrated methylation changes in promoters of various genes involved in obesity, appetite control and/or metabolism [18], such as *LEP* and *POMC* genes.

The LEP-coded leptin hormone has a direct effect on POMC by stimulating pro-opiomelanocortin expression in hypothalamic neurons, encoded by the *POMC* gene, and both are involved in a complex network of systemic signals and neural pathways that regulate food intake and energy balance [19-20].

In severely obese men and women, decreased levels of *LEP* methylation have been associated with increased levels of LDL-c, suggesting that it can regulate their epigenetic profile in adipose tissue. In addition, similar associations between *LEP* and LDL-c gene DNA methylation levels were observed,

suggesting a common regulatory route for DNA methylation in fat and blood [21]. Increased levels of *POMC* methylation were associated with altered serum lipids regardless of body weight in children [22].

In view of the above, the present unprecedented study proposed to evaluate the methylation levels of *LEP* and *POMC* genes with lipid profile values after intervention with dietary folate and hazelnut oil in overweight women.

Methods

This is a double-blind, placebo-controlled intervention study. Participants in the study were recruited from the population-based study entitled "II Cycle of Diagnosis and Intervention of the most Prevalent Food, Nutritional and Transmissible Situation of the Population of the Municipality of João Pessoa / PB (II DISANDNT / JP)" (COSTA 2016), conducted between May 2015 and May 2016. The study was approved by the Research Ethics Committee of CCS / UFPB, under protocol number 0569/15 and also submitted and registered in the Clinical Trials, under number NCT 02846025.

From the II DISANDNT/JP (COSTA 2016), data were originated that subsidized and continue to subsidize the conduct of research involving the information collected. In this study, based on inclusion and exclusion criteria, several articles were prepared both at population level [23–24] and at the level of dietary intervention and DNA methylation [9–25] or polymorphism [26–27] in several genes, always based on eligibility to participate in the study objectives.

Inclusion criteria for the study were: adult women, aged between 20 and 59 years, overweight or obese, (body mass index (BMI) 25.0–35.0 kg / m²), with different socioeconomic levels, drug users or not (except those who interfered with folic acid metabolism in the last three months), and with preserved cognitive function. The exclusion criteria were: alcoholism, smoking, neuropsychiatric disorders, use of drugs known to interfere with folic acid metabolism (in the last 3 months), use of multivitamin or mineral supplements, use of anorexics or anabolic substances, chronic diseases affecting the endocrine and metabolic system, pregnancy, pregnancy plans and weight loss during the study period.

After checking the eligibility criteria, 40 adult women were selected by convenience sample and were duly instructed on the study objectives according to the ethical guidelines. The women who agreed to participate in the study signed the informed consent form.

INTERVENTION PROTOCOL

Participants were instructed to maintain the weight, eating habits, and physical activity levels found during the initial evaluation [28] and were also given an individual diet plan 1 week before beginning the weight maintenance intervention. All drug treatments remained unchanged throughout the study. At the end of the study, all participants received nutritional counseling for weight loss.

To calculate energy expenditure, the DRIs (Dietary Reference Intake) formulas were used to maintain body weight. Macronutrients were distributed according to the recommendations of the American Heart Association (AHA) [29], and the calculation and analysis of nutrients present in the recommended diet were performed using the food equivalent system proposed by Costa [30]. The diet contained the following distribution of macronutrients: carbohydrates: 45–65% (recommended level of 55%), protein: 10–35% (recommended level of 15%) and total fat: 25–35% (recommended level of 30%).

Before the intervention began, the 40 participants were randomized en bloc using Stata® 13.0 software (College Station, Texas, USA) into four groups. Each group with 10 participants: group 1 (G1), in which participants received 300 g of vegetables and pulses containing an average of 191 µg / day of folate and 1 capsule of hazelnut oil (25 g); group 2 (G2), in which participants received 300 g of vegetables and pulses containing an average of 191 µg / day of folate and 1 capsule of placebo; group 3 (G3), where participants received 300 g of vegetables and legumes containing on average 94 µg / day of folate and 1 capsule of hazelnut oil (25 g); and group 4 (G4), where participants were only accompanied and maintained their regular eating habits.

The following foods containing higher concentrations of folate were used for each intervention group: lentils, soybeans, corn, peas, carrots, zucchini, lettuce, chard, beet, broccoli, cauliflower, tomato, and cucumber. The hazelnut oil capsule offered was basically composed of monounsaturated fat (68%), rich in oleic acid. Each group was composed of 10 women (as previously reported), who received vegetables and vegetables rich in folic acid daily for a total period of 8 weeks.

Anthropometric And Dietetic Measures

The body weight was measured in triplicate and the average of the three values was used. To evaluate the habitual food consumption of the individuals four 24 Hour Food Recalls (R24h) were applied, two before the beginning of the intervention with an interval of 15 days, having as objective the knowledge of the alimentary habits to subsidize the elaboration of the menu that was implemented and comparison of the consumption at the end of the study.

After the first week, the third R24h was applied to verify the adherence to the nutritional counseling proposed. And at the end of eight weeks the fourth R24h was applied to analyze the usual intake of calories and nutrients, in order to verify whether there was adherence to the recommendations and change in food consumption in general and folate after the intervention.

To assist in filling the R24HR, a photo album of foods with home measurements was used, this tool was based on the average food intake validated for the population of the study municipality, minimizing possible biases of this method [31–32]. Food consumption was analyzed by Dietwin nutrition software and then the multi-source method (MSM) was used to estimate the individual's regular intake of repeated measurements over a given period; the variation in intake was not affected by the method [33].

The other anthropometric and consumption variables were described in a previous study with another gene by LIMA et al. [9].

COLLECTION OF SAMPLES

The collection of blood at home, occurred respecting the criteria recommended for this purpose, performed at the residence of each participant before and after intervention, submitted to a fast of 12 hours, the blood collected from the brachial vein with the aid of a withers, positioned in the midline of the arm, maintained in sterile tubes of 4 mL under vacuum, with clot activator to analyze the serum and blood leukocytes, stored under conditions recommended in the literature [9], for analysis of the lipid profile and DNA methylation, respectively.

ANALYSIS OF LIPID LEVEL

The lipid profile, comprising total cholesterol, low density lipoprotein (LDL-c), high density lipoprotein (HDL-c) and triglycerides was analyzed using the serum, from the turbidimetry method in the Labmax 240 premium automated biochemical analyzer Labtest equipment. The calculation of LDL-C values was estimated by Friedewald, Levy and Fredrickson equation [34].

ANALYSIS OF THE METHYLATION LEVEL OF THE LEP AND POMC GENES

The DNA was isolated from the leukocytes using the QIAamp DNA Mini kit (Qiagen, Valencia, CA, USA) following the protocol described by the manufacturer. The genomic DNA was modified by the bisulfite conversion kit Cells-to-CpG™ (Applied Biosystems, Life Technologies, California) according to the manufacturer's instructions. PCR amplification and High-Resolution Melting (HRM) analysis was performed in the Applied Biosystems 7500 Fast System [9].

The PCR was performed in a total volume of 20µl containing: AmpliTaq® Gold 360 Buffer, 10x, 25mM Mg Chloride, Melt/Doctor™ HRM Dye (20x), 25nM from each primer with the primer sequences. For the *LEP* gene, Forward: 5'TGGAGAAGTGATGTTTTAA3' and Reverse: 5'ACCAAAACCCCTACTCTAAAATC 3' (167 bp), ring temperature (59 °C). For the *POMC* gene, Forward: 5'AGGAGGGTTTATTAATTTGTT 3' and Reverse: 5' ACTAACTCTCCAAACCAACC 3' (143 bp), ring temperature (59.8°C).

The sequence of the primers was obtained from the promoter region deposited in the Genome Browser database (<https://genome.ucsc.edu/>): *LEP*, chr7:128,241,278 – 128,257,629, 4 CpG sites, and *POMC*, chr2:25,160,853 – 25,168,690, 5 CpG sites, using the MethPrimer Software (<https://www.urogene.org/methprimer/>). The qPCR program consisted of an initial enzymatic activation at 95°C for 10 min, followed by 40 cycles of 15s at 95°C, 60s at 60°C and a final extension at 95°C 10s, 60°C 60s, 95°C 15s and 60°C 15s.

The fusion curves were normalized by calculating the 'best fit line' between two normalizing regions before and after the main fluorescence decrease representing the fusion of the PCR product using the Applied Biosystems 7500 Fast System software v 2.0.

STATISTICAL ANALYSES

Statistical analyses were performed with R software version 3.6.2. A comparison between the four groups was made using ANOVA (Analysis of Variance) for comparing means, when the assumptions of normality, independence and homoscedasticity of errors were being respected (to test such assumptions the Shapiro-Wilk, Durbin-Watson and Bartle test, respectively). When at least one of the assumptions was not being respected, the Kruskal-Wallis mean comparison test was used. Then the comparison was performed before and after intervention in each of the groups. Among the significant results, the t and Wilcoxon tests were used for paired samples for normal and non-normal data, respectively, to check for differences (between means) in the study variables before and after intervention in each group. As for data normality, the ShapiroWilk test was used.

To determine if there were significant relationships between the values of the methylation levels of *LEP* and *POMC* genes and the other variables of the study, the linear regression models described below were proposed:

Model 1: μ_{LEP} methylation levels gene = $\beta_0 + \beta_1 \cdot \text{Age} + \beta_2 \cdot \text{Total cholesterol} + \beta_3 \cdot \text{LDL-C} + \beta_4 \cdot \text{HDL-C} + \beta_5 \cdot \text{Triglycerides}$.

Model 2: μ_{POMC} methylation levels gene = $\beta_0 + \beta_1 \cdot \text{Age} + \beta_2 \cdot \text{Total cholesterol} + \beta_3 \cdot \text{LDL-C} + \beta_4 \cdot \text{HDL-C} + \beta_5 \cdot \text{Triglycerides}$.

The significance level adopted for all analyses was 5%.

Results

The sample consisted of 40 overweight women with the following characteristics: age, 44 ± 10.1 years, weight before intervention was 77.7 ± 14.1 kg and after intervention, 74.4 ± 14 kg ($p=0.40$), and BMI before intervention was 30.5 ± 5.3 kg/m² and after intervention, 29.2 ± 5.1 kg/m² ($p=0.37$) among all groups. Regarding the *LEP* gene methylation levels there was a decrease in the methylation levels ($p=0.02$), and in the lipid profile, where there was an increase in the HDL-c level ($p= 0.01$) (Table 1). In the comparison in each group, the weight loss was observed in group 2 ($p=0.00$), group 3 ($p=0.00$) and group 4 ($p=0.00$) (results not demonstrated).

Table 1. Anthropometric characterization, methylation levels and lipid profile of the sample before and after the intervention.

Variables	Before intervention		After intervention		p-value
	Mean	SD	Mean	SD	
Weight (kg)	77,7	14,1	74,4	14	0,40
Height (m)	1,59	1,1	1,59	1,1	-
BMI (kg/m ²)	30,5	5,3	29,2	5,1	0,37
<i>LEP</i> methylation (%)	47,27	14,23	40,22	13,58	0,02*
<i>POMC</i> methylation (%)	41,65	12,9	36,9	12,06	0,09
Total cholesterol (mg/dL)	201,6	46,2	194,8	48,1	0,58
HDL-c (mg/dL)	44,3	9,6	50,7	9,2	0,01*
LDL-c (mg/dL)	122,7	43,7	119,9	40,1	0,81
Triglycerides (mg/dL)	146,7	80,6	150,5	67,7	0,84

* t test

Also, in an analysis between the groups, it was found that the level of methylation for the *LEP* gene before intervention ranged from 45.7% to 49.20% and from 36.4% to 43.5% after intervention. The same was observed for the *POMC* gene in which the level of methylation before intervention varied from 38.7% to 43.6% and from 33.8% to 39.5% after intervention ($p > 0.05$), analyzed by the ANOVA test, for both genes there was no statistical difference before and after intervention between the groups ($p > 0.05$).

Regarding consumption before and after intervention, the means of the variables were compared among the four groups in this study and later only among the three intervention groups. There were statistically significant differences between folate consumption ($p > 0.00$) with larger differences in group 1 ($252.10 \pm 36.84 \mu\text{g}$) compared to the other intervention and control groups. Similarly, oleic acid ($p > 0.00$) and monounsaturated fat ($p > 0.00$) consumption in group 1 ($26.46 \pm 4.96\text{g}$, $33.35 \pm 9.28\text{g}$) showed significant differences compared to group 3 also supplemented ($33.37 \pm 8.57\text{g}$, $36.44 \pm 8.36\text{g}$), before and after the intervention, respectively, analyzed by the Kruskal-Wallis test. HDL-c levels ($p = 0.00$) in group 4 showed greater statistical differences ($57.80 \pm 7.88\text{mg/dL}$) compared to the three intervention groups (results not demonstrated), analyzed by the ANOVA test.

In an intra-group analysis, there was a decrease in methylation levels after intervention for the *LEP* and *POMC* genes in all groups, but for the *LEP* gene and the control group (G4) there was no statistical difference. Regarding the lipid profile, significant differences were found in groups G1, G3, and G4, where a decrease in cholesterol levels (G3 and G4), LDL (G1, G3, and G4), and triglycerides (G1 and G4) and an increase in HDL (G1, G3, and G4) were observed (Table 2).

Table 2. Description and comparison of the variables before and after the intervention in each group.

Group 1	Before	After	p-value
<i>LEP</i> methylation (%)	49,20 ± 14,78	43,50 ± 11,91	0,0085 ^{*1}
<i>POMC</i> Methylation (%)	43,50 ± 11,45	39,50 ± 9,97	0,0190 ^{*1}
Total Cholesterol (mg/dL)	189,50 ± 39,19	180,50 ± 34,84	0,0857 ¹
LDL-c (mg/dL)			
HDL-c (mg/dL)	121,60 ± 31,21	111,80 ± 28,58	0,0430 ^{*1}
Triglycerides (mg/dL)	38,90 ± 6,26	41,20 ± 6,30	>0,0000 ^{*1}
	169,10 ± 52,47	164,30 ± 47,27	0,0456 ^{*1}
Group 2			
<i>LEP</i> methylation (%)	47,60 ± 15,17	42,30 ± 16,41	0,0034 ^{*1}
<i>POMC</i> Methylation (%)	43,60 ± 14,29	39,30 ± 13,61	0,0190 ^{*1}
Total Cholesterol (mg/dL)	197,30 ± 40,26	199,40 ± 52,27	0,8428 ¹
LDL-c (mg/dL)			
HDL-c (mg/dL)	130,00 ± 34,78	124,30 ± 38,06	0,3333 ¹
Triglycerides (mg/dL)	42,90 ± 6,42	47,70 ± 9,52	0,0533 ¹
	133,60 ± 60,05	151,90 ± 59,46	0,0857 ²
Group 3			
<i>LEP</i> methylation (%)	46,60 ± 12,58	39,60 ± 12,46	0,0056 ^{*1}
<i>POMC</i> Methylation (%)	40,80 ± 13,70	36,40 ± 13,38	0,0109 ^{*1}
Total Cholesterol (mg/dL)	191,90 ± 65,40	181,40 ± 60,95	0,0012 ^{*1}
LDL-c (mg/dL)			
HDL-c (mg/dL)	122,90 ± 59,16	108,20 ± 56,91	0,0000 ^{*2}
Triglycerides (mg/dL)	40,60 ± 11,13	48,10 ± 8,38	0,0058 ^{*2}
	160,00 ± 80,52	141,00 ± 58,73	0,0676 ¹
Group 4			
<i>LEP</i> methylation (%)	45,70 ± 14,97	36,40 ± 14,31	0,0762 ¹
<i>POMC</i> Methylation (%)	38,70 ± 13,43	33,80 ± 12,19	

Total Cholesterol (mg/dL)			0,0176 ^{*1}
LDL-c (mg/dL)	191,90 ± 35,66	181,40 ± 60,95	
HDL-c (mg/dL)			0,0049 ^{*1}
Triglycerides (mg/dL)	146,00 ± 29,03	113,80 ± 25,64	
	49,60 ± 9,43	57,80 ± 7,88	0,0017 ^{*1}
	168,50 ± 99,88	143,20 ± 79,53	0,0004 ^{*1}
			0,0091 ^{*2}

G1: 191 µg / day of folate and 1 capsule of hazelnut oil (25 g); G2: 191 µg / day of folate and 1 capsule of placebo; G3: 94 µg / day of folate and 1 capsule of hazelnut oil (25 g); G4: no intervention; 1-test t; 2-test Wilcoxon; *statistically significant.

In table 3 it was verified which variables tested previously were associated with the methylation levels of both genes studied in the 3 intervention groups. An association between *POMC* gene methylation levels with lipid profile was observed in group 2 of the intervention, when the participants had higher *POMC* methylation levels they also had higher serum triglyceride values, that is, when triglyceride increased by 1 mg/dL the methylation levels increased 0.04%.

Table 3. Multiple regression analysis of *POMC* gene methylation level and lipid profile for group 2 of the intervention study.

Model 1 (*POMC* gene) / R-squared 0.6752

	Coefficient	95% CI	t statistic	p-value*
Age (years)	-0.88	2.4743 ± 0.6949	-1.56	0.19
Total cholesterol (mg/dL)	-0.04	-0.6725 ± 0.5745	-0.22	0.83
LDL-c (mg/dL)	-0.17	-0.7056 ± 0.3578	-0.91	0.41
HDL-c (mg/dL)	0.49	-1.8885 ± 2.8808	0.58	0.59
Triglycerides (mg/dL)	0.04	-0.0041 ± 0.8739	1.95	0.00*

*p < 0.05; Within this model, no significant relationships were observed between the *LEP* gene and lipid profile in the 3 intervention groups and for the *POMC* gene in groups 1 and 3 of the intervention.

Discussion

Previously we showed that a diet with folate and hazelnut oil was able to change the DNA methylation profile and the weight of obese women [9]. From this interesting result we felt encouraged to invest in studies in this population and understand the effect of the diet with folate and hazelnut oil on the

methylation profile of genes involved in lipid metabolism as well as the lipid profile in blood tissue. Thus, in the present study we show that this diet is associated with a decrease in the methylation level of *LEP* and *POMC* genes as well as with an improvement in the lipid profile.

The literature has shown that diet can in fact contribute to changes in the methylation profile of a variety of genes [9–14, 35].

In a previous intervention study with hypocaloric diet in obese women, a decrease in the level of methylation in the *LEP* gene promoter in fat tissue was observed, as well as weight loss and improvement in the lipid profile (reduction of total cholesterol) [36]. These data and the data from this study together show that the *LEP* gene is diet responsive in both fat and blood tissue [37], suggesting that leptin has its methylation levels reduced in metabolic disorders, in addition to being correlated with some important biochemical parameters and can serve as a marker of response to dietary intervention [38].

The hypocaloric diet used in the study of CORDERO et al. [36], however, was not associated with an improvement in triglyceride, LDL and HDL profiles as observed in the present study, particularly in the groups that received folate and hazelnut oil (G1 and G3), in a normocaloric diet. In the group that received only folate (G2), it is observed that there was no improvement in the lipid profile, suggesting that hazelnut oil may play an important role in this process [39–41].

Still in relation to G2, curiously, the parameters methylation profile and lipid profile were not altered concomitantly, that is, although a decrease in the *LEP* methylation profile was detected, no improvement in the lipid profile was observed, showing once again that the *LEP* gene is diet responsive and perhaps the lack of hazelnut oil in this group did not impact the lipid profile.

Regarding the *POMC* gene, the results were similar to those found for *LEP*, i.e., a decrease in methylation levels was detected in the groups with intervention with folate and hazelnut oil (G1 and G3) and only with folate (G2), also showing that the *POMC* gene is diet responsive. For our knowledge, there are no studies in the literature evaluating the effect of diet on the methylation profile in the *POMC* gene in obese women population. However, a study conducted on a population of men and women with normal weight, who underwent diet intervention with excess saturated and unsaturated fat showed that these people had increased weight as well as increased *POMC* promoter methylation level in fat tissue, but there was no change in triglyceride levels [42].

Interestingly, the non-intervention group (G4) also showed a decrease in the *POMC* methylation profile as well as an improvement in the lipid profile. These data can be justified by the conduct of the study and data collection at home involving nutritionists, as seen in the literature [43–45], although participants have committed themselves to maintaining dietary habits, the data suggest that there were changes in food consumption.

Association between methylation profile and lipid level was shown in a study conducted with severely obese people, in which it was observed that increased levels of methylation at different CpG sites in the

region promoting *LEP* in blood tissue and subcutaneous fat tissue was correlated with increased levels of LDL-c [21]. In vitro studies have shown that the incubation of endothelial cells with LDL increases the levels of DNA methylation by oxidized LDL into specific gene loci by inducing expression and activity of methyltransferase 1 DNA [46, 47], suggesting that LDL-c can modulate the DNA methylation profile.

On the other hand, a study showed that leptin (encoded by *LEP*) can regulate the metabolism of cholesterol ester (EC) through the activation of Hormon-Sensitive Lipase (HSL) in macrophages, an enzyme that performs the degradation of EC, thus protecting against atherosclerosis [48]. Thus, the origin of this association is still inconclusive, or even can be interdependent.

In an attempt to detect the association of the methylation profile with the lipid profile in the present study, a logistic regression was performed (Table 3), from which it was detected that the increase in the methylation level in *POMC* is related to the increase in triglycerides in the group that received only folate (G2). Similar results were reported between the higher level of *POMC* methylation (no exon 3) and significantly higher fasting triglycerides in children aged 7–9 years [49].

Our data show that although *POMC* has been diet responsive as observed by the decreased level of methylation (Table 2), the lack of hazelnut oil suggests that this supplement may play an important role in the modulation process of triglyceride level as well as in the DNA methylation profile of the *POMC* gene. It should be noted that G2 was the only group in which there was no improvement in the profile of any lipid studied.

Most scholars have reported associations between *POMC* gene variants and obesity phenotypes related to metabolic syndrome. However, recent studies do not make completely clear the mechanisms by which *POMC* methylation and lipid profile changes occur [22,49,50] and the role of dietary fat intake in DNA methylation profile changes [51–53].

It is known that the decrease in the level of methylation in the promoter is associated with increased expression of *LEP* [54] and *POMC* [55]. As verified from the genome sequences deposited in the Genome Browser, it is necessary to mention that the proximal promoter region of both *LEP* and *POMC* present a CpG island suggesting that DNA methylation can be a regulating factor of gene expression. In this sense, the decrease of the methylation levels found in the present study may be related to the increase of the expression levels and its measurement could help in the understanding of the relationship between the methylation levels, transcript level and lipid profile.

Nutrition is in fact a modifying factor of epigenetic marks and little is known about the biochemical mechanisms involved in these processes. It is believed that the main ways in which methylation can be altered by nutrition are: alteration of the substrate and co-factors that are necessary for the methylation reactions and alteration of the activity of enzymes that regulate the processes of methylation and demethylation of DNA [56].

Diet-gene interactions remain important determinants for lifelong health [57]. Our data show that both *LEP* and *POMC* are diet responsive with folate and hazelnut oil or folate alone and in addition it is suggested that intervention with both can enhance the improvement of the lipid profile. As limitations of the study, we cite the lack of expression analysis as well as dosage of co-factor levels for methylation, which could help to elucidate the relationship between processes.

Conclusion

This randomized, double-blind, placebo and controlled trial is the first to provide additional evidence of a normocaloric intervention with dietary folate and hazelnut oil supplementation on the levels of methylation in *LEP* and *POMC* genes and the role they can play in lipid metabolism. It has been observed in the literature that lipids induce changes in DNA methylation and changes in this process may impact genes and metabolism, this study highlighted the genes studied as important for the obesity and with a possible role in comorbidities such as dyslipidemia.

Declarations

Ethics approval and consent to participate

The study was approved by the Research Ethics Committee of CCS / UFPB, under protocol number 0569/15 and also submitted and registered in the Clinical Trials, under number NCT 02846025.

Consent for publication

The women who agreed to participate in the study signed the informed consent form.

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Availability of data and materials

The data generated in this study are coordinated Dr. Maria José de Carvalho Costa

Interest conflicts

The authors declare that they have no competing interest

Authors' contributions

T.R.C; R.P.A.L; R.C.P.L; F.E.L.L.F; M.C.R.G; A.T.C.A; R.M.M and A.S.S; were responsible for designing the study protocol, writing the paper; T.R.C; R.P.A.L and M.J.C.C were responsible for conducting the study;

N.F.P.O; C.W.P; D.H.N; R.T.L and M.J.C.C were responsible for analyzing data and interpreting results. All authors read and approved the final manuscript.

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