Effects of obesity, and of weight loss following bariatric surgery, on methylation of DNA from the rectal mucosa and in cfDNA from blood

Khalil ElGendy (khail.elgendy@nhs.net)
Human Nutrition Research Centre
Fiona Malcomson
Sorena Afshar
North Cumbria Integrated Care NHS Foundation Trust https://orcid.org/0000-0002-4676-8117
Mike Bradburn
Surgery Department
John C. Mathers
Newcastle University

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Abstract

Background

DNA methylation is an epigenetic mechanism through which environmental factors including obesity influence health. Obesity is a major modifiable risk factor for many common diseases including cardiovascular diseases and cancer. Obesity-induced inflammation resulting from aberrantly-methylated inflammatory genes may drive disease risk. This study is the first to investigate the effects of weight loss induced by bariatric surgery (BS) on DNA methylation in the rectum and in cell-free DNA (cfDNA) from blood.

Subjects and Methods:

DNA methylation was quantified in rectal mucosal biopsies and cfDNA from serum of 28 participants with obesity before and 6 months after BS, as well as in12 Non-Obese participants matched for age and sex from the Biomarkers Of Colorectal cancer After Bariatric Surgery (BOCABS) Study. DNA methylation of *LEP, IL6, POMC, LINE1, MAPK7* and *COX2* was quantified by pyrosequencing.

Results

BMI decreased significantly from 41.8kg/m^2 pre-surgery to 32.3kg/m^2 at 6 months after BS. Compared with Non-Obese participants, obesity was associated with lower *LEP* methylation in both the rectal mucosa and in cfDNA from serum. BS normalised *LEP* methylation in DNA from the rectal mucosa but not in cfDNA. BS decreased methylation of some CpG sites of *LINE*1 in the rectal mucosal DNA and in cfDNA to levels comparable with those in Non-Obese participants. Methylation of *POMC* in rectal mucosal DNA was normalised at 6 months after BS.

Conclusion

BS normalised *LINE1*, *POMC* and *LEP* methylation in the rectal mucosa of patients with obesity to levels similar to those in Non-Obese individuals. These findings support current evidence of effects of BS-induced weight loss on reversibility of DNA methylation in other tissues. The DNA methylation changes in the rectal mucosa shows promise as a biomarker for objective assessment of effects of weight-loss interventions on risk of cancer and other diseases.

Introduction

Obesity is a major modifiable risk factor for many common diseases including colorectal cancer (CRC) (1). Analysis of 56 observational studies by Ning *et al.*(2) revealed that each 5 unit increase in BMI was

associated with 18% increased CRC risk and participants with a BMI greater than 30 kg/m^2 (obese) had 41% higher CRC risk than those who were non-obese (BMI < 25 kg/m^2). Obesity increases cancer risk by multiple mechanisms, including adipose tissue dysfunction that may cause chronic inflammation and through increased insulin resistance which may increase proliferation and survival of CRC cells(3).

Bariatric surgery (BS) is an effective therapy which induces long-term weight loss and improves several comorbidities in obese patients(4). BS lowers the risk of cardiometabolic disease(5) and lowers incident cancer risk including CRC(6), which may be due to reduced inflammation in the rectal mucosa(7). These changes are also associated with decreased systemic and adipose tissue inflammation(8). However, the underlying molecular mechanisms though which BS-induced weight loss lowers CRC remain unresolved.

DNA methylation is an epigenetic mechanism that may mediate the impact of obesity on CRC risk. For example, within colonocytes, the methylation of oncogenes is modulated by obesity-induced inflammation(9). In a recent systematic review(10), we showed that BS can normalise DNA methylation at specific genomic locations in several tissues. However, none of the studies included in that systematic review reported the effects of BS on methylation of DNA in the rectal mucosa or of cell-free DNA (cfDNA) obtained from blood.

The aim of this project was to investigate the effects of obesity and BS on methylation at specific genomic loci in DNA from the rectal mucosa and in cfDNA from serum from participants in the Biomarkers Of Colorectal cancer After Bariatric Surgery (BOCABS) Study(11).

Subjects And Methods

The Biomarkers of Colorectal cancer After Bariatric Surgery (BOCABS) Study

The details of the BOCABS Study have been published elsewhere(11). In summary, the BOCABS Study investigated the impact of weight loss induced by bariatric procedures on biomarkers of CRC risk. Measurements and samples were collected prior to, and 6 months after, the bariatric procedure and compared with data from an age- and sex-matched non-obese healthy ‘Control’ group.

Adults (18-65 years) listed for a bariatric procedure at a single centre (North Tyneside General Hospital, UK) from November 2013 to November 2014 were approached by the research team after their pre-operative clinic visit. All candidates for surgery achieved 5% weight reduction following a 12-week multidisciplinary weight management programme. The Control group were non-obese adult participants (18-65 years, BMI <30 kg/m^2) who had undergone lower endoscopic examination within the last year without any significant colorectal pathology.

The BOCABS Study was conducted in accordance with the Declaration of Helskini and a favourable opinion was granted by the National Research Ethics Service Committee, North East - Newcastle & North Tyneside (13/NE/0204) on the 2nd of August, 2013. All study participants provided informed written consent. Caldicott Approval for Transfer of Identifiable Data/Information (C2700) was provided by

Northumbria NHS Healthcare Foundation Trust. The study was registered on the ISRCTN register (ISRCTN95459522).

Measurements and collection of biological samples:

At baseline assessment, demographic data and detailed medical history were recorded at face-to-face interview by S. Afshar. Anthropometric measurements (height, weight, waist and hip circumferences) and weight, BMI and body fat percentage (using a bio-impedance device; Tanita TBF-300MA body composition analyser) were measured. Blood samples were collected after a standard 6-hour fast using BD Vacutainer plastic serum tubes. Within 20 minutes of collection, samples were centrifuged at 3100g for 5 minutes. Serum was aspirated and stored in -80°C freezers for further analysis.

Ten rectal mucosal biopsies were collected circumferentially at 10cm from the anal verge during rigid or flexible sigmoidoscopy. No bowel preparation was used. Biopsies were collected using 2.3mm disposable flexible shaft biopsy forceps with spike (Medisafe International, UK). Samples were wrapped in aluminium foil and snap-frozen in liquid nitrogen for transport, then stored at -80°C.

Laboratory methods:

The 'GenElute Mammalian Genomic DNA Miniprep' kit (SigmaAldrich) was used to extract DNA from rectal mucosal biopsies following the manufacturer's protocol (12). Qiagen's DNeasy® Blood & Tissue Kit (Qiagen, UK) was used to extract cfDNA following the manufacturer's protocol(13). However, due to the extremely low concentration of cfDNA, certain steps were modified and optimised to achieve the necessary concentration of cfDNA in the final solution which are detailed below.

Bisulphite modification of DNA extracted from the rectal mucosal biopsies and of cfDNA was performed using the EZ DNA Methylation-Gold™ kit (Zymo Research) following the manufacturer's protocol with some optimisation to increase the cfDNA yield (please see Supplementary Material)(14). For cfDNA, changes were applied to the standard protocol of bisulphite conversion(14) to increase the final concentration of cfDNA: preparation of CT conversion: 700 µl of water, 300 µl M-dilution buffer and 50µl M-dissolving buffer were added into the CT conversion reagent tube and 110 µl of CT conversion reagent and 40 µl of the DNA sample were pipetted into a PCR tube. Bisulphite modified DNA (BM-DNA) was amplified using GoTaq® Hot Start Green Master Mix (Promega) with PyroMark® CpG Assays (Qiagen, Germany) and IDT-Primer assays (Integrated DNA Technologies Inc. USA) were used as primers for the amplification of BM-DNA and for pyrosequencing. For each assay, the optimal annealing temperature was determined after running the PCR amplification over a range of temperatures (48-60°C) followed by agarose gel electrophoresis (LiCor Odyssey imager).DNA methylation at each CpG site was quantified by pyrosequencing using the Pyromark Q96 ID (Qiagen) Pyrosequencer and Pyromark Gold Q96 reagents (Qiagen) using the manufacturer's protocol.

Details of the assays for each gene are summarised in Supplementary Table 1. In addition, supplementary Table 2 summarises information about the genetic loci that were investigated. In

summary, *LINE1* was chosen as a surrogate for global DNA methylation(15), while *IL6*, *LEP*, *COX2, MAPK7* and *POMC* where selected as genes linking obesity, inflammation and CRC risk.

Reproducibility and Quality Control:

All analyses included negative controls to check for contamination. Any samples indicating incomplete bisulphite conversion were flagged by the Pyromark software at the analysis stage and were consequently excluded from subsequent analyses. All samples were run in duplicate. If the results for duplicates of a given sample were within 5% of each other, the values were included in the analysis. If the methylation measurements for duplicates were not within 5% of each other, the assay was repeated. If the difference between the results was still higher than 5%, either the closest of two readings (with a difference less than 5%) or the average of four readings was included (if the difference was 5-10%). If the difference was more than 10%, the results of that sample were excluded. On each pyrosequencing plate, representative samples from each subgroup (control, pre-surgery and post-surgery) were included to minimise potential batch-to-batch bias.

Statistical methods:

Descriptive statistics were used to summarise the demographic and clinical characteristics of participants and levels of DNA methylation. Prior to further analyses, the distribution of datasets was checked for normality using the Shapiro-Wilk test. Where data were not normally distributed ($p<0.05$), non-parametric tests were used.

The control (non-obese) and pre-surgery (obese) groups were compared using analysis of variance (ANOVA; general linear model, univariate analysis) with sex, age and BMI as covariates. Sensitivity analysis of the results was done by comparing the results from ANOVA against p-values generated from independent student t-test (parametric) or Mann Whitney U test (non-parametric), as appropriate. Pre- and post-surgery groups were compared using paired T-test (parametric) and Wilcoxon signed ranks tests (non-parametric).

Results

Twenty-eight initially obese patients completed the BOCABS Study from whom samples and data were collected before, and at 6 months after, surgery. In addition, measurements and biological samples were taken from 12 non-obese participants (Control group) matched for age and sex (Table 1). Before surgery, the mean BMI of the obese group was in the morbidly obese range (41.8 kg/m^2) and this fell substantially to mean 32.3 kg/m^2 at 6 months post-surgery. There were corresponding falls in other obesity markers including body weight, fat percentage and waist circumference (Table 1). Note that these participants remained, on average, obese and that their BMI was significantly greater than that of the non-obese controls. Blood concentrations of high sensitivity C-reactive protein (hs-CRP) and of leptin were significantly reduced at 6 months after BS but remained higher than in the Non-Obese Control group.

Obesity alters patterns of DNA methylation in the rectal mucosa

Methylation of *IL6* and *LEP* was higher in DNA from rectal mucosal biopsies in the Non-Obese group (Control) compared with the Obese group (Pre-Surgery). For *LEP*, this was statistically significant for CpG sites 3 and 4 and for the mean of all CpG sites (52.3% vs 46.9%) (Fig. 1B). In contrast, methylation of *LINE1*, *POMC* and *MAPK7* was lower in DNA from rectal mucosal biopsies in the Non-Obese group compared with Obese Pre-Surgery individuals. This finding was statistically significant for methylation of all CpG sites in *LINE1*, for CpG site 4 and the mean of all CpG sites (3.4% vs 5.7%) in *POMC* and for CpG sites 2 and 4 in *MAPK7*.

No methylation was detected at CpG site 1 in *POMC*, at CpG sites 4, 5 and 6 in the second primer of *MAPK7* and at all CpG sites in the second primer of *COX2* in DNA from rectal mucosal samples. Consequently, these genomic loci have been excluded from the analysis.

Bariatric surgery normalises DNA methylation of LEP, LINE1, POMC and MAPK7 in the rectal mucosa

At 6 months post-BS, methylation of all CpG sites, except CpG site 2, in *LEP* increased and methylation of *LINE1*, *POMC* and *MAPK7* decreased (Fig. 1). These weight loss-related changes were statistically significant for all CpG sites in *LINE1* (Fig. 1D), CpG site 4 and the mean of all CpG sites (7% vs 8.3%) in *POMC* (Fig. 1F) and CpG sites 3 and the mean of all CpG sites (14.2 vs 14.8%) in *MAPK7* (Fig. 1C). We observed no significant differences between DNA methylation levels in rectal mucosa from the Post-Surgery group and the Non-Obese group for any gene except *MAPK7.* Methylation of CpG sites 2 and 4 in *MAPK7* was significantly higher in the Obese group Post-Surgery than in the Non-Obese Controls (Fig. 1C). There were no significant changes in methylation of *IL6* and *COX2* following BS.

Obesity is associated with altered methylation of cfDNA

When compared with the Non-Obese (Control) group, methylation of *LINE1* and *MAPK7* was higher in Obese individuals Pre-Surgery. This increase was statistically significant for CpG site 5 in *LINE1*. On the contrary, methylation of *IL6* and *LEP* was significantly lower in the Obese Pre-Surgery group compared with the Non-Obese Controls at all CpG sites investigated, except for CpG site 1 in both *IL6* and *LEP* (Fig. 2A and 2B).

Bariatric surgery normalised methylation of LINE1, IL6 and LEP in cfDNA from blood

At 6 months after BS, methylation of *LINE1* and *LEP* in cfDNA had fallen compared with values Pre-Surgery. This observation was statistically significant for CpG sites 3 and 5 and for the mean of all CpGs sites (73.9% post-surgery vs 75.3% pre-surgery) of *LINE1* (Fig. 2D) and for CpG site 4 for *LEP* (Fig. 2B). On the contrary, methylation of *IL6* in cfDNA was higher at 6 months after BS, and significantly so for CpG site 2 (Fig. 2A).

Discussion

This is the first investigation of the effects of obesity, and of weight loss following BS, on DNA methylation in the rectal mucosa and in cfDNA. As summarised in Table 2, obesity was associated with altered methylation of *LINE1* (an index of global DNA methylation (15)) and of a panel of genes linking obesity, inflammation and CRC risk including *IL6, LEP, POMC* and *MAPK7*. These alterations were reversed (normalised) following weight loss induced by BS (Table 2). For *LINE1*, associations with obesity and changes in response to BS observed in DNA from rectal mucosa were paralleled by differences in cfDNA. However, DNA methylation patterns in rectal mucosal DNA and in cfDNA were less consistent for the other genes in our panel.

Associations between obesity and DNA methylation

The lower methylation of *LINE1* in DNA from rectal mucosal biopsies (CpG sites 2 and 4) and in cfDNA (CpG site 5) in Non-obese participants is contrary to what we expected since *LINE1* hypomethylation is associated with higher risk of CRC(16). In a study of methylation of DNA from white blood cells in young (mean age 23 years), apparently healthy individuals (n = 156), Marques-Rocha *et al.*(17) found that indicators of adiposity, such as skinfold thickness and total body fat, were lower among individuals with higher *LINE-1* methylation. There was no difference in *LINE1* methylation in cfDNA from blood between obese and non-obese individuals in the BOCABS Study. This observation was similar to the findings from two studies that investigated *LINE1* methylation in whole blood and in leukocytes. Nicoletti *et al.*(18) reported no difference in *LINE1* methylation in blood between 36 obese and 9 healthy controls. A similar finding was reported by Duggan *et al.*(19) who investigated *LINE1* methylation in leukocytes from 300 obese participants and 59 controls.

On the contrary, methylation of *LEP* in both DNA from rectal mucosal biopsies and in cfDNA was higher in the Non-Obese group compared with obese participants. This observation is supported by findings from House *et al.*(20) who observed a negative association between BMI and *LEP* methylation in blood from 73 obese patients. Similarly, Obermann-Borst *et al.*(21) reported that higher BMI was associated with lower methylation of *LEP* in blood from 120 children.

There was no significant difference in *IL6* methylation in DNA from rectal mucosa samples between Pre-Surgery Obese and Non-Obese participants. However, *IL6* methylation was higher in cfDNA from Non-Obese individuals. IL6 is a key regulator of inflammation and higher concentrations are associated with obesity and chronic inflammation(22). If methylation of *IL6* is associated with transcriptional silencing(23), lower levels of *IL6* methylation would be expected to lead to higher expression and higher circulating concentrations of IL6 in obese individuals. However, Carraro *et al.*(24) reported that higher *IL6* methylation in blood was associated with higher BMI. The apparent conflict between these observations may relate to the specific CpG sites at which methylation was quantified since not all CpG sites within the same genomic region respond in the same way to environmental influences(25).

Methylation of *POMC* in DNA from rectal mucosal biopsies was lower in Non-Obese than in Obese participants. This finding is in line with those by Kuehnen *et al.*(26), who found that *POMC* methylation, quantified in 171 obese and 90 normal weight adolescents, was hypermethylated in obese individuals.

Given evidence that methylation may inhibit *POMC* expression(27), one would anticipate that the lower methylation in non-obese individuals would result in higher *POMC* expression. POMC has anti-inflammatory properties and is associated with reduced risk of CRC(28). Thus, higher methylation in the rectum of obese participants, if associated with increased *POMC* expression, could be a potential mechanism for higher CRC risk in those living with obesity.

MAPK7 encodes a kinase that is part of the MAPK pathway that regulates cell proliferation, differentiation and apoptosis(29) and is a candidate oncogene(30). In the present study, *MAPK7* methylation was lower in non-obese than in obese individuals, which is contrary to what is expected as *MAPK7* hypomethylation has been observed in CRC(31). These apparently conflicting observations could be reconciled if hypomethylation of *MAPK7* occurred later in the oncogenic process and so would not be detectable in macroscopically normal tissue, as investigated here.

Effects of bariatric surgery on DNA methylation:

Our previously systematic review showed that 12 out of 15 included studies reported significant changes in DNA methylation after BS in diverse tissues, including blood and adipose tissues (10). However, none of the studies investigated DNA methylation in rectal mucosal samples or in cfDNA from blood.

LEP methylation in rectal mucosal samples from BOCABS participants was significantly higher 6 months after BS and BS normalized methylation levels of *LEP* in the rectal samples, as demonstrated by the similarity in *LEP* methylation between post-surgery obese and non-obese individuals. It is important to note that the BMI of the obese group 6 months after BS was still within the obese range (mean = 32.3 kg/m^2) in comparison with the won-obese group (mean = 25.3 kg/m^2). These changes in *LEP* methylation in the rectal mucosa might contribute to the protective effects of weight loss in decreasing risk of CRC. Since *LEP* is overexpressed in CRC, higher *LEP* methylation leading to reduced *LEP* expression may lower risk of CRC(32). However, these favourable changes were not observed in cfDNA where methylation of *LEP* was lower after BS. This might be explained by differential effects of BS on metabolic and inflammatory outcomes in different body systems which can affect *LEP* methylation in cfDNA(33).

BS also normalized *POMC* methylation in rectal biopsies after 6 months in comparison with non-obese participants. If this lower *POMC* methylation is associated with increased expression, this would be expected to lower CRC risk since POMC has anti-inflammatory properties(28).

LINE1 methylation was lower in the rectal mucosa at 6 months after BS in obese patients with no significant difference between the post-surgery obese and non-obese groups, suggesting that *LINE1* methylation was restored to non-obese levels. Similarly, methylation of *LINE1* in cfDNA from blood was reduced after BS. These findings differ from the results of studies included in our systematic review and meta-analysis (10), where no effects of BS on *LINE1* methylation in blood were observed. Martin Nunez *et al.*(34) and Nicoletti *et al.*(18) did not find any changes in *LINE1* methylation in whole blood or buffy coat, respectively, 6 months after BS. This might be explained by the difference in methylation between sample

types (DNA from blood which is largely from DNA within cells versus cfDNA from blood). From a wider perspective, the finding of lower *LINE1* methylation in non-obese individuals and in the post-surgery group compared with the obese group pre-surgery is contrary to what might be expected since *LINE1* hypomethylation has been linked to increased CRC risk(16). The reasons for this unexpected finding remain to be investigated.

There was no significant change in *IL6* methylation after BS in DNA from rectal mucosal biopsies but methylation of *IL6* in cfDNA increased after BS. In our published meta-analysis(10), the effects of BS on IL6 methylation differed between individual studies with reports of no effect(35), decreased methylation(18) or increased methylation(36) of *IL6* in blood after BS leading to no overall effect in the meta-analysis. These heterogeneous findings may due to differences in participant characteristics, in the follow-up duration, and in the degree of weight change achieved after BS in each of the studies. In addition, the specific effects on BS on different tissues are likely contributing factors to the differential effects on *IL6* gene methylation across studies.

Conclusions

BS normalised *LINE1*, *POMC* and *LEP* methylation in the rectal mucosa of patients with obesity to levels similar to those observed in Non-Obese individuals. These findings support current evidence that BS-induced weight loss can reverse aberrant patterns of DNA methylation seen in other tissues. The DNA methylation changes in the rectal mucosa shows promise as a biomarker for objective assessment of effects of weight-loss interventions on risk of cancer and other diseases.

Declarations

Competing interests

The authors declare no competing financial interests.

Data availability statement

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Authors' contribution:

KE is the corresponding author, responsible for writing the manuscript, conduct the laboratory experiments, extracting and analysing data, interpreting results and creating figures and tables. FCM assisted in laboratory experiments, analysing data writing the manuscript, laboratory work, analysing data, writing and revising the manuscript. SA designed and conducted the BOCABS study including ethical submission, recruitment and biological specimen collection. MB: contributed to feedback and final revisions of the manuscript. JCM: supervised the BOCABS study, including patient recruitment and

sample collection, supervised laboratory work, provided feedback on the results and data analysis and contributed to the feedback and final revisions of the manuscript.

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Tables

Table 1: Demographic and anthropometric data of participants in the BOCABS Study

	Non-Obese (n=12)	Obese (n=28)		P-value	
		Pre-surgery	Post-surgery	Control vs Pre-Surgery	Pre- vs Post-Surgery
Female: n (%)	7 (58%)	23 (82%)		0.133[1]	NA
Age (years)	51.5 (21-61) †	46.6 (7.98)		0.36[2]	NA
Diabetes	8.3% (n=1)	25% (n=7)		0.35[1]	NA
Weight (kg)	73.47 (14.96)	114.8(16.46)	88.6(16.4)	<0.001[3]	<0.001[5]
BMI (kg/m2)	25.3 (0.72)	41.8 (5.84)	32.3 (5.37)	0.009[3]	<0.001[5]
Body fat percentage	30.27 (4.48)	49.55 (33.8 -65.6)†	38.25 (7.23)	<0.001[2]	<0.001[4]
Waist circumference (cm)	88.7 (12.27)	123.3 (13.43)	98.6 (16.8)	<0.001[3]	<0.001[5]
hsCRP (mg/L)	1.05 (0.2-11.7)†	4.75 (0.9-19.4)†	1.05 (0.2–7.6)†	0.034[2]	<0.001[4]
Leptin (ng/mL)	6.75 (1-38) †	45.76 (1.3-308)†	13.9 (1-108)†	0.008[2]	0.001[4]

Parametric values are expressed in mean (SD), while non-parametric values (†) are expressed in median (range), [1]: Chi Square test, [2]: Mann-Whitney test, [3] : Independent T-test, [4]: Wilcoxon test, [5]: Paired T-test .

Table 2: Summary of significant findings of associations with obesity and effects of BS on DNA methylation in the rectal mucosa and in cfDNA from participants in the BOCABS Study

Gene	Associations with obesity		Effects of bariatric surgery	
	Rectal mucosa	cfDNA	Rectal mucosa	cfDNA
LEP	↓ (CpG 3,4 and mean)	↓ (CpG 2, 3,4 and mean)	↑ (CpG 3,4 and mean)	↓ (CpG 4)
LINE-1	↑ (CpG 2, 4 and mean)	↑ (CpG 5)	↓ all CpG sites	↓ (CpG 3,5 and mean)
IL6	↔	↓ (CpG 2 and mean)	↔	↑ (CpG 2)
MAPK7	↑ (CpG 4)	↔	↓ (CpG 3 and mean)	↔
POMC	↑ (CpG 4 and mean)	NA	↓ (CpG 4 and mean)	NA
COX2	↔	NA	↔	NA

↓ decreased methylation, ↑ increased methylation, ↔ no change, NA: not available,

Figures

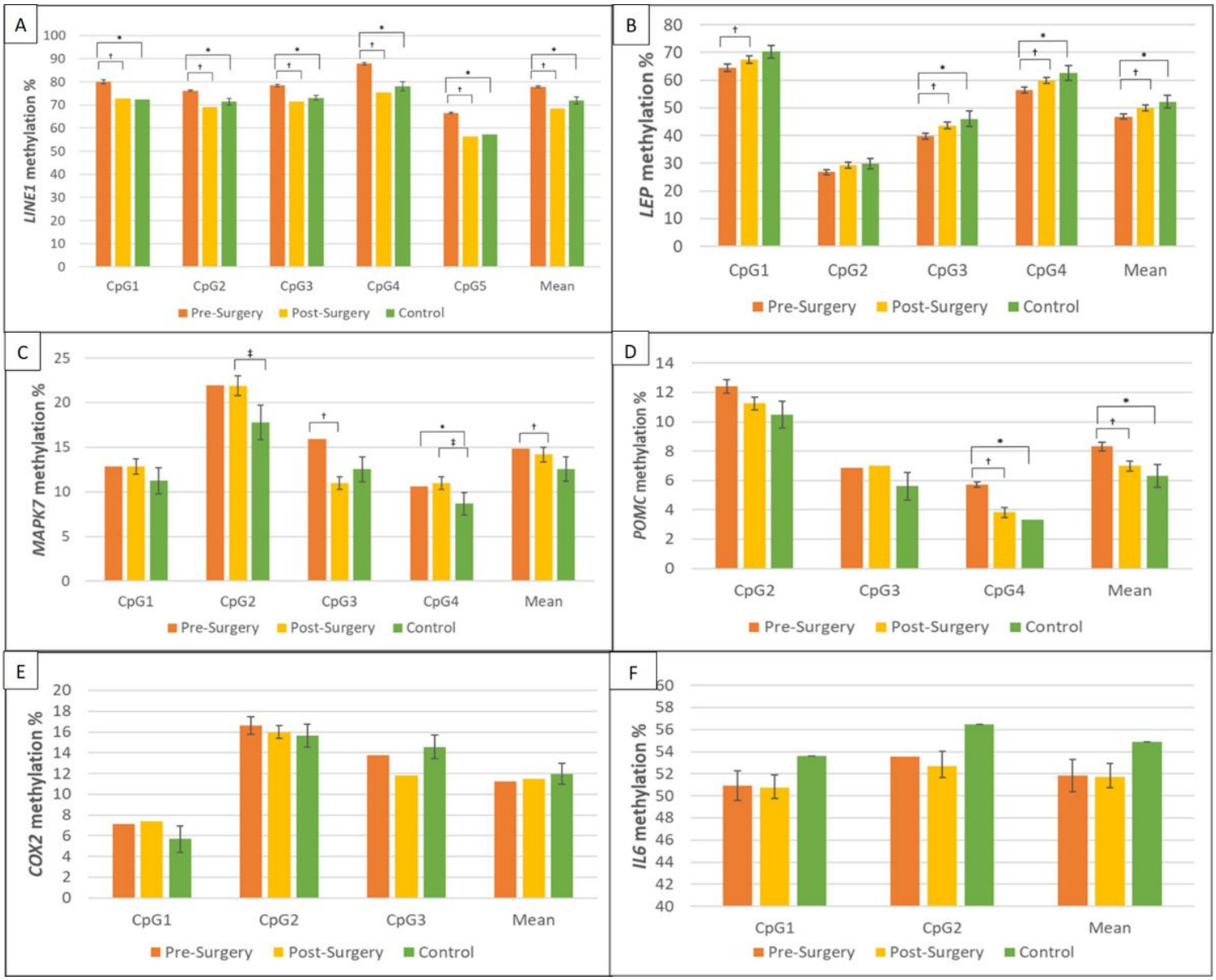

Figure 1

Methylation (%) at specific genomic loci in DNA from rectal mucosal biopsies obtained from obese participants Pre-Surgery and at 6 months Post-Surgery and from non-obese Controls. A) *LINE1;* B) *LEP;* C) *MAPK7*, D) *POMC;* E) *COX2* F) *IL6*

*: p-value <0.05 when comparing Pre-Surgery Vs Non-Obese (Control)

†: p-value <0.05 when comparing Pre-Surgery Vs Post-Surgery

‡: p-value <0.05 when comparing Non-Obese (Control) Vs Post-Surgery

Mean and SEM are shown when the data were normally distributed and parametric analyses were used and Median value is shown when the data were not normally distributed and a non-parametric test was used.

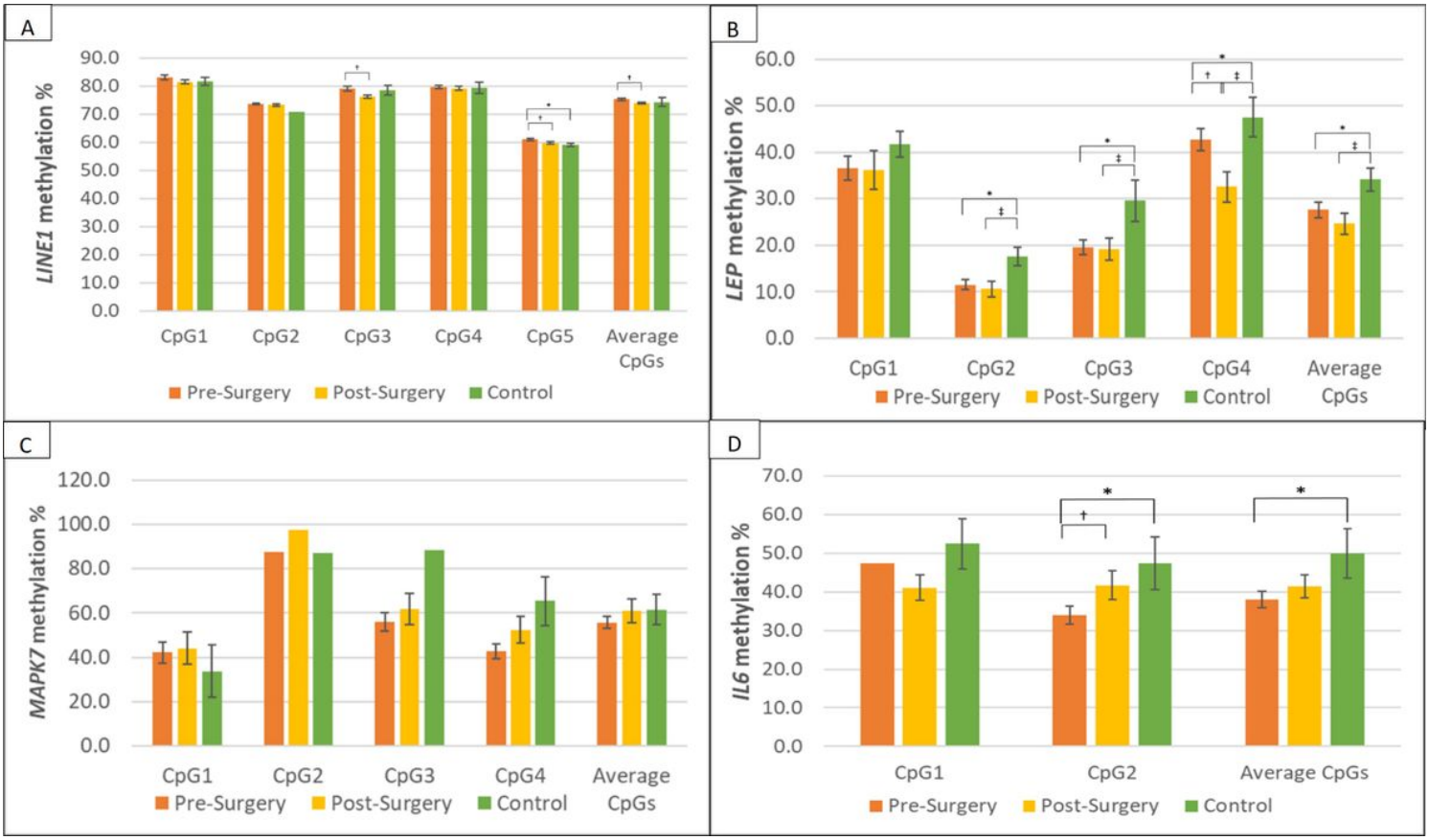

Figure 2

Methylation (%) in cfDNA from serum at the following specific loci: A) *LINE1* B) *LEP* C) *MAPK7* D) *IL6*

*: p-value <0.05 when comparing Pre-Surgery Vs Non-Obese (Control)

†: p-value <0.05 when comparing Pre-Surgery Vs Post-Surgery

‡: p-value <0.05 when comparing Non-Obese (Control) Vs Post-Surgery

Mean and SEM are shown when the data were normally distributed and the Median is shown when the data were not normally distributed.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterials.docx