

Brief Report

Serum Cobalt Concentration and DNA Methylation Signatures in Women with Obesity

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Abstract: Obesity, a multifactorial disorder, has been associated with alterations in metal metabolism and epigenetic modifications. This pilot case–control study aimed to investigate serum cobalt concentrations and associated DNA methylation patterns in women with obesity. Serum cobalt levels were measured using inductively coupled plasma mass spectrometry (ICP-MS), revealing significantly higher cobalt concentrations in participants with normal weight than in participants with obesity. Additionally, DNA methylation analysis identified differentially methylated positions (DMPs) associated with cobalt exposure, and DMPs between groups highlighted hypomethylation in the top DMPs in individuals with obesity. Functional enrichment analysis of these DMPs unveiled potential pathways implicated in apoptosis, cancer, and metabolic signaling, warranting further investigation into the mechanistic links. This study provides preliminary insights into the interplay between cobalt exposure, DNA methylation, and potential implications for obesity management.

Keywords: cobalt; DNA methylation; obesity; inductively coupled plasma mass spectrometry; epigenetics

1. Introduction

Obesity is a significant risk factor for chronic diseases, including cardiovascular and heart diseases. Rates of obesity are growing in adults and children. The percentage of adults with obesity more than doubled from 7% to 16% globally [1]. The prevalence of obesity in the US was 41.9% in 2017 until 2020, according to the CDC [2]. Obesity is a global health concern characterized by excessive fat accumulation resulting from complex interactions between genetic, environmental, and lifestyle factors [3].

While the etiology of obesity is multifaceted, recent studies have implicated environmental factors in its pathogenesis, including a deficiency of essential minerals. Minerals can alter glucose’s metabolic inflammation and endocrine control and may be associated

with obesity [4]. A cross-sectional study found a correlation between the level of dietary minerals and the odds of obesity in childhood, including Fe, Zn, Cu, and Na intake [5].

Metals are dispersed in the environment (via soil, water, air, and dust), consequently being dispersed in the human food chain and in the water [6]. Cobalt, a transition metal, is ubiquitous in the environment and is found in various industrial processes, dietary sources, and medical implants [7]. Cobalamin, known as Vitamin B12, is a natural, non-toxic, environmentally friendly cobalt complex. It is a member of the broader cobamide family that all contain the same corrin ring with a cobalt ion ligand. The relationship between the cobalt ion and the corrin ring is important with respect to its biological function, including DNA synthesis and cellular regulation [8]. Emerging evidence suggests the potential role of cobalt exposure in metabolic dysfunction and obesity development. Levels of pollutants and cobalt were associated with lower BMI. Copper and manganese were found to have strongly positive associations with obesity status, and cobalt was found to have negative associations with obesity status [9,10]. An epidemiological study observed that cobalt was inversely associated with body mass index (BMI) and waist circumference [11,12].

Heavy metals can induce epigenetic alterations and disrupt the epigenome. Recently, 38 studies focused on epigenetics and metal-induced neurotoxicity, highlighting potential epigenetic mechanisms in etiology [13]. However, the relationship between cobalt and obesity is rarely explored in an epigenetic context. Furthermore, epigenetic mechanisms, particularly DNA methylation, have been implicated in obesity susceptibility and metabolic dysregulation. This case-control study aimed to analyze the cobalt serum concentration in women with obesity and check the DNA methylation signatures associated with this metal, as well as the most differentially methylated positions in patients with obesity that were correlated with this metal.

2. Materials and Methods

2.1. Population and Sampling

This is a pilot case-control study. A total of 33 Brazilian women participated in this study, 17 with normal weight and 16 with obesity. Adult women with no other diagnosed diseases, non-smokers, and those who did not consume alcoholic beverages participated in this study. The evaluations occurred at the Hospital das Clínicas de Ribeirão Preto of the University of São Paulo (HCRP-USP). It is worth noting that the sample selection was made by convenience.

2.2. Ethical Aspects

The HCRP-USP ethics committee approved this study, with CAAE license number 14275319.7.0000.5440. All participants were informed about the study's objective and signed the research participation form. We adopted the principles of the Declaration of Helsinki for this study. This clinical study is registered under the universal trial number U1111-1267-7108.

2.3. Epigenetic Analysis

Total leukocyte DNA was converted to bisulfite using the EZ DNA Methylation Kit (Zymo Research Corporation, Irvine, CA, USA). After conversion, 500 ng were hybridized with the Infinium Human Methylation 450k BeadChip (IHM450K Illumina, Inc., San Diego, CA, USA). After following the protocol recommendations, we obtained the images using the iScan system. We extracted intensities using Genome Studio (Illumina, Inc., San Diego, CA, USA). This BeadChip contains genic and intergenic regions [14], and all targets can be verified in the manufacturer's documentation.

2.4. Bioinformatic Analysis

We performed the analysis using R version 3.6.2 and the ChAMP package available in Bioconductor [15]. The raw intensity data from the IHM450K idats files were loaded into the R package, ChAMP, and normalized by cell type using the function myRefBase

<- champ.refbase [16]. We then used the DMP <- champ.DMP function to calculate linear regressions to verify the identification of differentially methylated positions (DMPs) [17] associated with exposure to cobalt. The DMPs between groups were recovered by the limma function initially available in the Champ Package. Functional enrichment analysis of DMPs was conducted using the String database to elucidate the biological pathways impacted by cobalt-induced DNA methylation changes.

2.5. Cobalt Assessment

The determination of the total concentration of cobalt (Co59) in serum was performed by an inductively coupled plasma mass spectrometer (ICP-MS), fitted with a dynamic reaction cell (DRC) (Perkin Elmer Sciex Norwalk, CT, USA), following the protocol previously described [18]). The spectrometer was an ICP-MS ELAN 6100 Sciex® (PerkinElmer Instruments, Ribeirão Preto, SP, Brazil). Samples were diluted in a ratio of 1:50 with a solution containing Triton X-100 0.01% (v/v), HNO₃ 0.05% (v/v), and 10 mg/L rhodium (Rh) as an internal standard. The concentration of the analytical calibration standards ranged from 0 to 50 µg/L.

2.6. Statistical Analysis

We performed statistical analysis using SPSS software (v 25.0, Chicago, IL, USA). The Shapiro–Wilk test was adopted as a normality test. We adopted the Student’s T or Mann–Whitney test due to the normality presented in the data. These tests were adopted to verify the differences between the means between the groups, and we considered $p < 0.05$ as significant.

3. Results

Table 1 shows that serum cobalt levels were significantly higher in participants with normal weight compared to participants with obesity ($p < 0.05$).

Table 1. Clinical parameters.

Essential	Obesity (n = 16)	Normal Weight (n = 17)	Reference
Age	38.6 ± 11.6	39.1 ± 13.4	-
BMI	45.1 ± 5.4 ^a	22.6 ± 1.9	Obesity: BMI ≥ 30 kg/m ² Normal weight: 18.5 > BMI < 25 kg/m ²
Co (µg/L)	0.46 ± 0.08 ^a	0.64 ± 0.14	0.30–1.20 ^b

Co: Cobalt. ^a: significant difference ($p < 0.05$). BMI: body mass index. Age was not different between groups.
^b [19]

DNA methylation analysis revealed differential methylation patterns between patients with obesity and those with normal weight ($n = 3329$, Figure 1). There were 839 sites associated with cobalt, and 143 sites overlapped with DMPs Ob. and DMPs Cobalt (Figure 1). Table 2 shows information about the cobalt-related sites, and Table 3 highlights the DNA methylation of the top DMPs. They were predominantly hypomethylated in promoter regions. Functional enrichment analysis identified several KEGG pathways enriched for the genes presented in Table 3. We hypothesize that these genes enriched from cobalt-associated DMP annotation involve different pathways, including apoptosis, cancer, and metabolic signaling pathways.

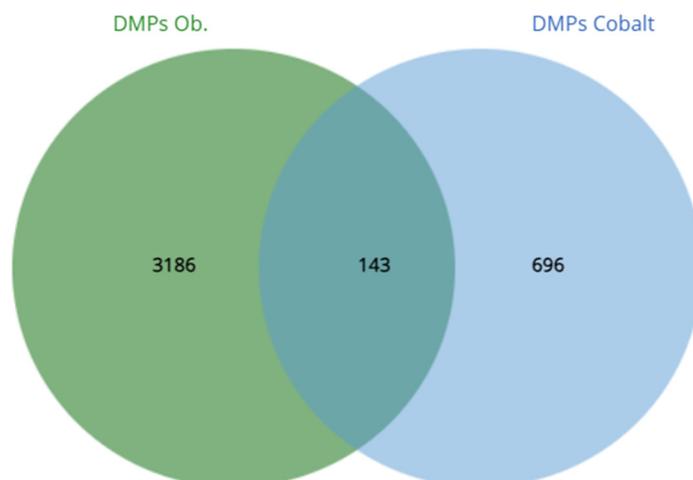


Figure 1. DMPs related to obesity and cobalt. NOTE: DMPs Ob contain 3329 DMPs, differentially methylated in individuals with obesity, and DMPs Cobalt are the DMPs related to cobalt. Those values were retrieved by Limma function and linear regression, respectively.

Table 2. Cobalt-related Differentially Methylated Positions (DMPs).

Metal	DMPs (Effect)	GR (%)	DHS Enhancer BOTH	FDR1	Genes (n)	Analysis	Termescription	Term ID	FDR 2
Cobalt	839	728 (86.8%)	143	4.2×10^{-3}	712	Keywords	Methylation	KW-0488	0.0024
			92				Chromatin regulator	KW-0156	0.0272
			21				Transcription regulation	KW-0805	0.0392

Note: Co: cobalt. DMP: differentially methylated position, obtained by linear regression analysis, $p < 0.050$, $FDR < 0.050$ (Benjamini–Hochberg analysis). GR (%): genic region percentage. DHS: DNase hypersensitive sites. FDR1: false discovery rate (FDR) in top differentially methylated positions (DMPs). Genes (n) = number of mapped genes. Term ID: enrichment identification term. FDR2: enrichment-generated false discovery rate (FDR) from each database. KW: keyword. KEGG: Kyoto Encyclopedia of Genes and Genomes.

Table 3. Top common CpGs independently associated with cobalt, differentially methylated in patients with obesity. Located in the promoter region.

Metal	CpG ID	CHR	Gene	Feature	$\Delta\beta$	p-Value	Methylation Status	p-Adjusted Value
Cobalt	cg16020249	1	AHDC1	TSS1500	−0.02	<0.001	Hypomethylated	0.048
	cg16108132	10	ANXA7	TSS1500	−0.02	<0.001	Hypomethylated	0.048
	cg02951206	3	MED12L	TSS1500	−0.02	<0.001	Hypomethylated	0.031
	cg09658576	7	TBXAS1	TSS200	−0.02	<0.001	Hypomethylated	0.016
	cg22280792	17	ENGASE	TSS1500	−0.02	<0.001	Hypomethylated	0.049
	cg02860394	2	RNF103	TSS200	−0.02	<0.001	Hypomethylated	0.038
	cg19921261	10	C10orf125	TSS200	−0.02	<0.001	Hypomethylated	0.025
	cg05010219	10	PWWP2B	TSS1500	−0.02	<0.001	Hypomethylated	0.046
	cg13175981	1	MCL1	TSS1500	−0.02	<0.001	Hypomethylated	0.012
	cg00986800	3	FNDC3B	TSS200	−0.02	<0.001	Hypomethylated	0.008
	cg23057220	19	MUM1	TSS200	0.07	<0.001	Hypermethylated	0.048
	cg24127861	14	REC8	TSS1500	0.06	<0.001	Hypermethylated	0.044
	cg10477989	11	C11orf24	TSS200	0.05	<0.001	Hypermethylated	0.012

Note: CpG ID: Identification number for each CpG site, CHR: Chromosome, $\Delta\beta$: difference between the beta values of normal weight group vs. group with obesity. TSS1500: Transcription Starting Site 1500 (refers to promoter region), TSS200: Transcription Starting Site 200 (refers to promoter region).

4. Discussion

The study participants showed a significant difference in BMI—the group of patients with obesity was classified as grade III obesity, while the normal weight group was placed

into the eutrophic classification based on BMI. Arain and Neitzel, 2019, reported a serum cobalt range of 0.3–1.2 µg/L, and both groups had cobalt levels within the expected range [19]. However, there was a difference between serum cobalt levels in the groups of patients with obesity and those with normal weight, with higher levels in patients with normal weight. Some studies reported a negative correlation between cobalt and obesity. Therefore, patients with a high BMI had lower levels of cobalt, suggesting that cobalt levels can be a risk factor for the development of obesity [9,11,20]. Inorganic cobalt appears to be preventive in obesity-related diseases by increasing leptin, adiponectin, and HDL [21]. In addition to the negative correlation with obesity, another study showed a negative correlation between urinary cobalt and insulin resistance [22].

This pilot study provides preliminary evidence of an association between serum cobalt concentrations, DNA methylation patterns, and obesity. The observed hypomethylation of specific genomic regions suggests a potential mechanism through which cobalt exposure may influence metabolic processes underlying obesity. Moreover, the enrichment of cobalt-associated DMPs in pathways related to apoptosis and cancer highlights the complexity of cobalt's effects on cellular homeostasis and suggests potential implications for obesity-related comorbidities. Further research incorporating larger sample sizes and longitudinal designs is warranted to validate these findings and elucidate the mechanistic links between cobalt exposure, epigenetic alterations, and obesity development.

Excess body fat is associated with epigenetic signatures in patients with obesity and its metabolic consequences [23,24]. Our study found 3329 obesity-related DMPs. Furthermore, the findings involved signatures, with the epigenome related to cobalt (839 DMPs), with 143 DMPs being common between obesity and cobalt. One study in the literature reported a relationship between cobalt and DNA methylation in pregnant women [25], and another study reported a protective effect of high levels of cobalt in plasma via AgeAccel, Horvath [26]. However, no studies have evaluated the influence of cobalt on DNA methylation in patients with obesity.

The presence of cobalt in this study was associated with CpG sites, suggesting a potential role in modulating DNA methylation. It was highlighted that cobalt, particularly in the form of methylcobalamin, a vitamin B12 derivative, acts as a cofactor for enzymes involved in DNA methylation [27]. The lower concentration of cobalt observed in individuals with obesity was hypothesized to contribute to abnormal DNA methylation patterns. It was suggested that different forms of cobalt exposure, including dietary intake and environmental contamination, could influence DNA methylation and subsequently impact various biological pathways [28].

Functional enrichment analysis revealed that cobalt-associated CpG sites were related to methylation, chromatin, and transcription regulation processes. It was suggested that cobalt might influence methylation patterns, potentially affecting chromatin structure and gene expression regulation [29].

This study discussed potential health implications of cobalt-induced DNA methylation alterations, including effects on apoptosis, necrosis pathways, cancer, and metabolic pathways. The findings suggested that cobalt exposure, particularly in certain forms, might exert endocrine-disrupting effects and influence cellular processes related to disease development [30,31]. Overall, the discussion highlighted the importance of considering cobalt exposure to DNA methylation patterns and its potential implications for human health, particularly obesity and metabolic disorders.

The impact of cobalt chloride (CoCl₂) on obesity has been demonstrated in the study by Kawakami et al. (2012) [32], which showed that cobalt significantly affects white adipose tissue in mice fed a high-fat diet, reducing adipocyte size and weight. Cobalt administration was also associated with increases in serum leptin, adiponectin, and HDL cholesterol levels, as well as the normalization of glucose levels. These effects suggest the beneficial role of cobalt in improving metabolic health markers and activating AMPK in key tissues, potentially offering a preventative approach to obesity-related diseases. This finding is in

stark contrast to the adverse effects noted with mercury, highlighting cobalt's therapeutic potential in obesity management [32].

Further evidence of cobalt's role in obesity management comes from Tetsuka et al. (2022) [33], who found a significant inverse correlation between urinary cobalt levels and BMI within the Tokyo Teen Cohort, suggesting that cobalt may reduce obesity risk in early adolescents, particularly males. This underscores the need for additional research to verify these outcomes and explore the mechanisms involved [33].

It is hypothesized that the lower levels of cobalt in individuals with obesity may be related to the quality of their diet. Cobalt, primarily known for its crucial role in the formation of vitamin B12, is vital for nerve function, DNA production, and red blood cell formation. Vitamin B12, which contains cobalt, is found in significant amounts in animal products such as meat, fish, dairy, and eggs. Although only small amounts are required, cobalt's presence in the diet via vitamin B12 is essential for healthy metabolic processes, including the synthesis of fatty acids and energy production. Ensuring an adequate intake of foods rich in vitamin B12 is important for maintaining sufficient cobalt levels to support these critical bodily functions [7].

The sample size in this pilot study and the lack of data regarding the nutritional intake of cobalt are a limitation and may restrict further adjustments, but to eliminate this bias, we only included women to avoid possible sexual effects in relation to cobalt exposure. The strengths of this study are the extensive analysis of DNA methylation and its link to cobalt in obese and normal-weight women, presenting new epigenetic insights into cobalt exposure. The findings of this study are relevant for the Brazilian population and the world, as developing countries end up being more susceptible to the presence of toxic metals in drinking water.

5. Conclusions

This pilot case-control study provides preliminary evidence suggesting an association between serum cobalt concentrations, DNA methylation patterns, and obesity in women. The findings underscore the need for further investigation into the potential role of environmental exposures, such as cobalt, in modulating epigenetic mechanisms underlying obesity pathogenesis. Understanding the interplay between cobalt exposure, DNA methylation, and obesity may have important implications for preventive and therapeutic strategies targeting obesity and its associated complications.

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Data Availability Statement: Data will be partially available upon request.

Conflicts of Interest: The authors declare no conflicts of interest.

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