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Research Article

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Epigenetic Regulation by Epigallocatechin-3-Galatte on Antioxidant Gene Expression in Mice That Are at High Risk for Heart Disease

Banzubaze, E[1]*, Ondari, E. N[2], Aja, P. M[3], Shinkafi, T. S[3], Mulindwa, J[4], Ochwo, S[5], Wampande, E[5], Ndabarushimana, L[6], Ngezahayo, J[7], Bivuzimana, P. J[8], Muvunyi, R[9], Nibogora, C[10]

[1]*Department of Paraclinical Sciences, Institut National de Santé Publique (National Institute of Public Health). Bujumbura, Burundi*

[2]*School of Pure & Applied Sciences, Department of Biological Sciences, Kisii University*

[3]*Faculty of Biomedical Sciences, Department of Biochemistry, Kampala International University-WC, Ishaka, Uganda.*

[4]*Department of Biochemistry and Sports Science, School of Biosciences, College of Natural Sciences, Makerere University. Kampala, Uganda.*

[5]*Department of Bio-molecular Resources and Bio-lab Sciences, School of Bio-security, Biotechnical and Laboratory Sciences, College of Veterinary Medicine, Animal Resources & Bio-security, Makerere University, Kampala, Uganda.*

[6]*Department of Mathematics and Computer Sciences, University Cheikh Anta Diop of Dakar. Dakar, Senegal.*

[7]*Research Centre in Natural and Environmental Sciences, Faculty of Sciences, University of Burundi, P.O Box 2700 Bujumbura, Burundi.*

[8]*Department of Paramedical Sciences, Université Panafricaine Les Mages (Pan-African University Les Mages), Bujumbura, Burundi.*

[9]*Department of Food Sciences and Nutrition, Faculty of Agronomy and Bioengineering, University of Burundi. Bujumbura, Burundi.*

[10]*Department of Paraclinical Sciences, Institut National de Santé Publique (National Institute of Public Health). Bujumbura, Burundi.*

***Corresponding Author**
Banzubaze, E, Department of Paraclinical Sciences, Institut National de Santé Publique (National Institute of Public Health) Burundi.

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Abstract

Cardiovascular disease (CVD) is the leading cause of mortality worldwide. Green tea-derived epigallocatechin-3-gallate (EGCG) has pathological and physiological processes on human, particularly CVD, and its significant role in epigenetic regulation. The study focused on the effect of EGCG on a high-fat diet (HFD) and a sedentary lifestyle (SL) and methylation of antioxidant genes. Samples from the liver tissues of male mice fed a high-fat diet (HFD) or two control diets (CD): a normal or standard diet and a low-fat diet, with or without EGCG supplementation. DNA

methylation involved subjecting male mice to a sedentary lifestyle or two control modes of physical exercise: voluntary physical exercise and chronic physical exercise with or without EGCG supplementation. The results revealed that the low-fat diet and chronic exercise caused DNA hypomethylation in the Sod2, Gpx1, Cat, and TrxR1 genes, while the HFD and the SL without EGCG showed DNA hypermethylation. EGCG supplementation led to a decrease in DNA methylation, also known as hypomethylation. Additionally, there was DNA hypermethylation as a consequence of the HFD diet and sedentary lifestyle, while EGCG supplementation reduced DNA damage due to hypomethylation. The results demonstrate that EGCG, on one hand affects DNA methylation associated with food components and a sedentary lifestyle, which affects the health of the host. On the other hand, the effects may come from antioxidant activities as well as epigenetic modifications observed in CpG methylation, but they are also likely to include other epigenetic elements. The results show that bioactive antioxidants can change the epigenome, mainly by fixing DNA methylation patterns that aren't working right, which potentially could stop oxidative stress. If we understand the underlying processes of these targets, we can strategically administer antioxidant substances and DNA methylation-modifying medicines to prevent and treat chronic disease and its associated comorbidities.

Keywords: Epigallocatechin-3-Gallate, High Fat Diet, Sedentary Lifestyle, Cardiovascular Disease, DNA Methylation, Epigenetics

1. Introduction

Cardiovascular disease (CVD) is the leading cause of mortality worldwide and includes coronary, congenital, and rheumatic heart disease, as well as peripheral arterial disease and chronic heart failure (CHF) [1]. Studies have demonstrated the multiple effects of Epigallocatechin-3-gallate (EGCG), derived from green tea, on human pathological and physiological processes, particularly CVD [2, 3]. EGCG is also very important in epigenetic regulator. For example, it can stop class I histone deacetylases (HDACs) from increasing the amount of acetylated histone and stop DNA methyltransferase from turning on genes that have been silenced by methylation [4–6].

Polyphenols and flavonoids inhibit DNA methylation by suppressing the activity of DNA Methyltransferases (DNMT) [7]. EGCG at a low concentration prevented DNA damage [8]. Epigenetic changes, such as post-translational modifications to the histone tails of nucleosomes, DNA methylation, and non-coding RNA regulation of chromatin remodeling, mediate many environmental factors that lead to CVD [9]. CVD epigenetics may represent such a field as well as a source for new therapeutic targets. External factors such as lifestyle, diet, and likely pharmaceutical medications modulate epigenetic changes [10]. Extensive research has examined the impact of diet and physical activity on CVD outcomes [11–12], yet there is still a lack of evidence regarding the mechanisms through which dietary components could potentially reversibly manipulate the epigenome of aging vascular cells. Indeed, this can suggest a significant role for epigenetic mechanisms in CVD diagnosis and therapy [13]. Epigenetic changes are dynamic features that modulate genome functionality under exposure to exogenous factors, providing a molecular pattern that maintains stable propagation of gene expression profiles from one cell generation to the next [14]. More and more proof shows that DNA methylation plays specific roles in managing the expression of key genes in atherosclerotic vascular tissues, which in turn affects CVD [15].

However, the epigenetic mechanism by which EGCG contributes to heart failure or CVD prevention remains unclear. The point of this study was to find out what kind of effects EGCG might have on a high-fat diet (HFD) and a sedentary lifestyle (SL). They also wanted to find out if EGCG could fix wrong DNA methylation patterns and boost antioxidant defenses, which would then stop or slow down CVD in mice. The study found that HFD and SL raised the levels of DNA methylation at CpG islands for the genes Sod2, Gpx1, Cat, and TrxR1. The study also revealed that EGCG triggers DNA hypomethylation, thereby increasing endogenous antioxidant defense.

2. Materials and Methods

2.1.1 Ethical Clearance

The animal ethics committee of Kampala International University and the Uganda National Council of Science and Technology authorized the methods and protocols used in this study; their approval number is NS 645. The treatment of animals complied with the US National Institute of Health Guide to the Care and Use of Laboratory Animals (NIH Publication N°.85-23, revised 1996) and the European Council of the Animal's Guidance in the Care and Use of Experimental Animals [16].

2.1.2 Experimental Set-Up

A total of sixty-three-month-old male Swiss albino mice from the animal facility of the College of Veterinary Medicine, Animal Resources, and Biosecurity (COVAB) of Makerere University were purchased. Mice were kept in typical cages, subjected to a 12-hour light/dark cycle, and kept at regular conditions of humidity and temperature (27 ± 2°C) and humidity (55 ± 5%), respectively. We created twelve groups, each consisting of five mice. The first six groups received physical exercise treatment as previously described by Banzubaze et al., 2022 [17,18] and Banzubaze et al., 2024 [19], while the remaining six groups received diet treatment as previously described by Banzubaze et al., 2022 [17,18] and Banzubaze et al., 2024 [19]. EGCG was dissolved in drinking

water and administered it at a daily dose of 30 mg/kg/day. The mice underwent euthanasia and anesthesia, following the previously described methods by Golab G.C. et al. [20], ILAR (2014) [21], and Grandjean [22], as cited by Banzubaze et al. [17-19]. We took tissue samples for DNA extraction, DNA methylation, and DNA methylation quantification analysis.

2.1.3 Determination of DNA Methylome

2.1.3.1 DNA Extraction and Quantification

Total DNA was extracted using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Catalogue N° 69504). In summary, we cut up about 5 mg of liver tissue samples and placed them in a 1.5 ml microcentrifuge tube. We added 20 l of proteinase K, vortexed the mixture, and let it sit at 56°C. Then, 200 µl Buffer AL was added and vortexed, and 200 µl Ethanol (96–100%) was added and vortexed again. We pipetted this mixture into a column in a 2 ml collection tube, centrifuged it at 6000 x g (8000 rpm) for 1 minute, and discarded the outflow. This reaction was repeated using 500 µL of Buffer AW1. Again, 500 µL of Buffer AW2 was added and centrifuged for 3 min at 20,000 x g (14,000 rpm); the outflow was discarded. Then, the column was placed in a clean 2 ml microcentrifuge tube, 200 µl of Buffer AE was added, and it was incubated at room temperature for 1 min. Next, we centrifuged it at 6000 x g for 1 minute. We quantified the resulting DNA using NanoDrop 2000C (Thermo Scientific, USA).

2.1.3.2 DNA Methylation by Bisulfite Method

2.1.3.2.1 Preparation of CT Conversion Reagent

Briefly, CT Conversion Reagent was prepared by adding 750 µL of water and 210 µL of Dilution Buffer M to a tube of CT Conversion Reagent, then vortexed for 1-2 minutes for a total of 10 minutes, and stored at 20°C in a dark room since it is sensitive to light. We thawed the reagent and vortexed it for 2 minutes at room temperature before use.

2.1.3.2.2 Bisulfite Treatment

Grandjean et al. previously described performing DNA methylation on bisulfite-converted DNA to determine the methylation status of sites CpG across the genome [22]. In summary, we added 5: l of Dilution Buffer M to the 500 ng - 1 g (14l) of sample DNA and adjusted the mixture to a total volume of 50 l with water. We pipetted the sample up and down for mixing. We incubated the sample at 37°C for 15 minutes. After the above incubation, 100 µl of the prepared CT conversion reagent was added to each sample and mixed. We then incubated the samples in a thermal cycler for 20 cycles, each at 95°C for 30 s and 50°C for 15 min, before completing a final hold step at 4°C. 400 µl of Binding Buffer M was added to a Zymo-SpinTM IC column, and the column was placed in a collection tube provided. We loaded the sample from the previous step onto the Zymo-SpinTM IC column containing binding buffer M. We closed the cap and mixed the sample by repeatedly inverting the column. We centrifuged the resultant sample at 11,000 x g for 30 seconds and discarded the continuous flow. Next, we added 200 l of M-Wash buffer to the column and centrifuged it at 15,000 rpm for 30 seconds. We added 200 l of desulfonation buffer M to the column and let it stand at ambient temperature (20–30°C) for 15-20 minutes. Following the incubation period, we centrifuged the sample at full speed (15,000 rpm) for 30 seconds. 200 µl of M-Wash buffer was added to the column, and a third centrifugation at full speed for 30 seconds was performed. This was repeated after the addition of 200 µl of M-Wash buffer and recentrifuged under similar conditions. We placed the column in a 1.5-ml microcentrifuge tube, directly added 20 l of pre-warmed Elution Buffer M to the column matrix, and incubated it at room temperature for 1 min. We then performed another 30-second centrifugation at full speed to elute the DNA. We repeated this last step with the same elution buffer, and this DNA was now ready for PCR.

2.1.3.2.3 DNA Methylation Quantification

Ehrich et al. (2005) described the use of the 5-mCDNA ELISA kit (Zymo Research) to measure DNA methylation [23]. We amplified the CpG regions by PCR using the primers shown in Table 1. We reconstituted the reaction mixture as indicated in Table 2 and incubated it at 37°C for 2 hours. The mixture was then washed three times with 5M NH4Cl and once with 100 mM citric acid. It was then mixed again in 10 µl of NH4 citrate and left to sit at 37°C for about 30 minutes.

The EpiTYPER assay (Sequenom, San Diego, CA) quantified gene-specific DNA methylation, following the previous description by Bibikova et al. [24]. The UCSC Genome Browser (http://genome.ucsc.edu/) showed that certain areas were good for DNA methylation [23]. These areas were found in our genes of interest with bisulfite-specific primers (Table 1) needed for the assay and to find out the methylation status of CpG sites across the genome, as explained by Grandjean et al. (2014) [22].

Gene	Forward Primer	Reverse Primer
Sod2	GGGTTTGGTTAATAAGATTGTTTTTT	TACAACTCTCTTCCACTTTACCCAA
Gpx1	TGGGAGTTTAGAGTTTAAGGTTATTT	AATCTCAACACACCATCCAATTAAAAA
Cat	GGGTTTGGTTAATAAGATTGTTTTTT	TACAACTCTCTTCCACTTTACCCAA
TrxR1	TTTTAAATTTTGATTTAGTTGGGGT	CCTTAAATCCATTTCCATCTAATTCT

Table 1: Primers used for methylation of the CpG Islands

Component	Reaction mixture (20 µL)
Taq DNA polymerase	0.1 µL
DNA	2 µL
T7 Forward primer	2 µL
SP6 Reverse primer	2 µL
Transcription reagent	10 µL
Nuclease free water	3.9 µL

Table 2. Reaction Mixture for DNA Methylation Quantification

2.1.3.2.4 DNA Data Methylation Analysis

We used the MassARRAY Compact mass spectrometer and Agena real-time detection software to analyze the data and find the cleaved products by mass spectrometry. In summary, we configured the plate using the EpiTYPERTM Plate Editor software. We imported DNA references and DNA names into Plate Editor to configure the plate with amplicons, samples, and reactions. This can be done on any PC that has the correct version of EpiTYPER software installed and is connected to the Sequenom database at the customer site or a Sequenom Service lab. Once the software identifies the plate as a 96-well chip, we use the Plate Editor software on the RT-Workstation to export the plate definition. This creates an XML file in the folder. You can double-click the Start RT Processes button in SpectroACQUIRE to open the RT-Workstation software on the C:/DataProcesses/Input disk. Next, open the "Auto Run Setup" tab and copy the experiment name from the C:/DataProcesses/Input folder from the previous step into the corresponding chip position field. To verify the correct export of the chip, click on the "Barcode Report" button. If the report displays "found," proceed to the next step, ensuring you have checked the option "Auto additionally, confirm that you have selected the MassCLEAVE parameter file for analysis. Set the acquisition parameters to Shots (N) = 12, Maximum Acquisitions = 5, Minimum Good Spectra = 5, and Maximum Good Spectra = 5. In the "Autorun" tab, click the "Start Autorun" button.

3. Results

3.1 DNA Methylation Targets PCR-Product

These lines show the DNA methylation analysis profile for the Sod2, GPX1, CAT, and TrxR1 genes in the CpG Island regions (Figure 1). The methylation by quantification approach did not reach the maximum level of uncertainty, resulting in undetectable methylation levels in some samples. Green colors for CpG Island regions are regions that were targeted during DNA methylation analysis; red colors are CpG Island regions that were not analyzed during DNA methylation, probably due to the high or low mass of these regions, and the underlined CpG Island sequences are assayed in one fragment. By looking at the results of these identification fragments of methylated or unmethylated CpG islands, we were able to find out how many CpG islands were found, how many were methylated, how many were unmethylated, and what percentage of CpG islands were methylated (Table 3).

The results of the identification of methylated CpG islands showed that the Gpx1 gene was most methylated, followed by the TrxR1 gene, and lastly, the Sod2 gene. This means that the Gpx1 and TrxR1 genes are more sensitive to environmental stimuli, such as diet and exercise. The increased methylation of DNA at the level of the Gpx1 and TrxR1 genes also means that when mice are exposed to severe dyslipidemia from things like HFD or SL, the antioxidant capacity of the enzymes encoded by these genes is negatively affected. This lowers the expression of Gpx1 and TrxR1, which in turn lowers the body's natural antioxidant defense. This creates a pathological state that can lead to CVD in the long term.

CAT

GGGCCTGGCCAACAAGATTGCCTTCTCCGGGTGGAGACCGCTGCGTCCGTCCCTG

CTGTCTCACGTTCCGCAGCTCTGCAGCTCCGCAATCCTACACCATGTCGGACAGTC

GGGACCCAGCCAGCGACCAGATGAAGCAGTGGAAGGAGCAGCGGGCCTCGCAG

GTACCCCGTGTTCCCCAGAGCCTCACGAGAATGTGGGCGGCGGCGAGTAACGGC

GCGGGCTTCCCTTGGGTGGCCTGCGCTGAGGCTGCACCACTGGCTGCGCTCGAGT

GGCTGCTTTCTGTCCCACGAACCGTTCCATTCTGCGGAGAGGGGATGGGACTGCG

GGCTTACAGCTTTTCGCTGGCAGCAGGGGCCGGGTGGATGGCTCCGAGTTTGGCA

TTTGTGCCAGCTGGGACATAGGTCACAGCTTAAGGGAGACATGGAGCCGTCTGGG

CAAAGTGGAAGAGAGCTGCA

SOD2

GGCAGGAGGCTGGGCCCTGTGGCCGGTGCCGCGGGCTCCCGGCACAAGCACAGC
CTCCCAGACCTGCCTTACGACTATGGCGCGCTGGAGCCACACATTAACGCGCAGA
TCATGCAGCTGCACCACAGCAAGCACCACGCGGCCTACGTGAACAATCTCAACGC
CACCGAGGAGAAGTACCACGAGGCTCTGGCCAAGGGTAGGTGCGGGGCCCCTGC
ACACGAGGATCCCGGTGGCCGGACGGAGGTCTGTTTCGATGCCGGGGAATACCTC
CGTTGGTTCGCATAAACGTCCTTTCCTATCCCGGAAAACACAGGACCTCACGAAA
GTACTTGACGGACCTTACTGTTGTGTCCTTTTTTGTCACCCGTGTTGCTAGCTAGC
ATTGGTGGTGGTCCT

TRXR1

TCCTAAATCCTGATTCAGTTGGGGCACGGGAAGGGGGGAGTGTCACAGGAGGAC
CCCGGTCCATCTCTTCACCTCCGCCAGATTTGGGCTGGCGGTAGGGAGTGCAGCT
CCGGGGACTCAGGTGCTGCCCACGAGTGCATCTCCCGTACCCGGCGTTCCAGGGT
CCCCTCGTCCCCTCCCCCAGGGCATGCTCCACCGCCGAGGGCGCACACAGGCCCT
GGGCCGCTAAGCCCGCGGGGACACCGAGAGTGTCCTCTTCAGCACCGCGGGATG
TGCGCGGCGCCCGGGCATGCGTACCTTGCTCTCGATCAGCTTCACCATTTTGGCTG
TTGCGGGGAGGGAGCCACACGCGTCTCAGCAGAACCAGATGGAAATGGATCCAA
GG

GPX1

GATCTCAGCACCATCCAGTTAAAAGGAGGTGCAGGGCCCTGTGAGCGCTAGTAC
GGATTCCACGTTTGAGTCCCAACATCTCCAGTATGTGTGCTGCTCGGCTCTCCGCG
GCGGCACAGTCCACCGTGTATGCCTTCTCCGCGCGCCCGCTGACGGGCGGGGAGC
CTGTGAGCCTGGGCTCCCTGCGGGGCAAGGTGCTGCTCATTGAGAATGTCGCGTC
TCTCTGAGGCACCACGATCCGGGACTACACCGAGATGAACGATCTGCAGAAGCG
TCTGGGACCTCGTGGACTGGTGGTGCTCGGTTTCCCGTGCAATCAGTTCGGACAC
CAGGTATATGGGGCGAGATGGGTGGCCTTGGGCTCTAGGCTCCCA.

Key: Green: Analysed, Red: Cannot be analyzed, underlined: assayed in one fragment.

Figure 1: Methylated CpG Islands after PCR Amplification

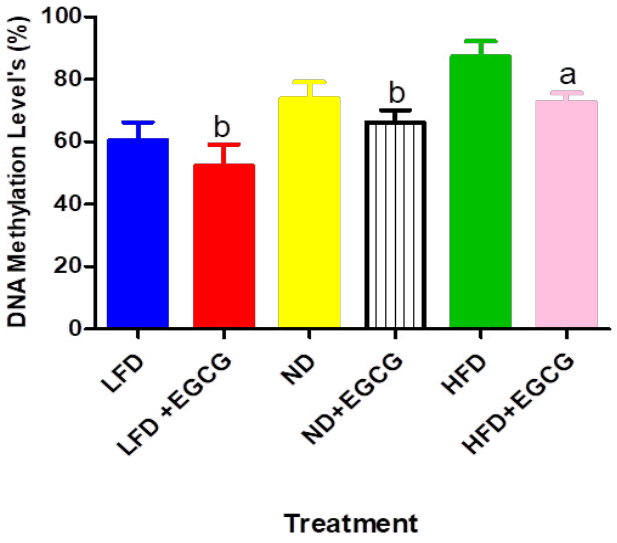
Gene	CpG islands identified	CpG islands methylated	CpG islands unmethylated	% of methylation levels
Sod2	29	18	11	62.06
Gpx1	27	25	2	92.59
Cat	30	20	10	66.67
TrxR1	28	25	3	89.28

Table 3: Identification of CpG Island regions

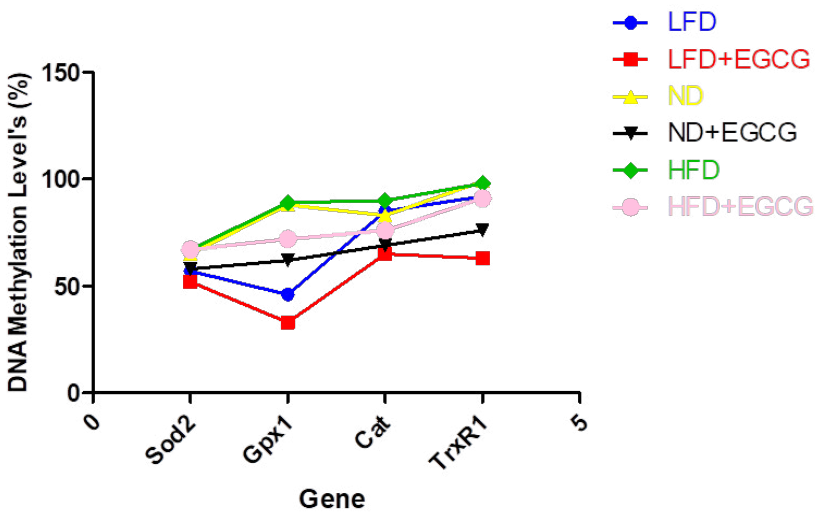
3.2 Effect of EGCG ON DNA Methylation Level After Diet Treatment in Mice

Figure 2A shows the connection between the effects of EGCG in food and the levels of DNA methylation in the Sod2, Gpx1, Cat, and TrxR1 genes. The p-value (0.0001) is less than 0.05, which means that the results show that there is a statistically significant link between EGCG added to mice's food and DNA methylation levels on the genes Sod2, Gpx1, Cat, and TrxR1.

These same results show that a diet high in fat increases DNA methylation (hypermethylation) at the level of genes involved in antioxidant defense. Supplementing with EGCG results in a decrease in the level of methylation, also known as hypomethylation (Figures 2A and B). We observed that an HFD supplemented with EGCG exhibited less methylation (hypomethylation), whereas an HFD without EGCG exhibited high methylation (hypermethylation). In mice with or without EGCG supplementation, there is a statistically significant link between food treatments and DNA methylation levels; the p-value is less than 0.0001. The Sod2 gene exhibited a low level of DNA methylation, followed by the Cat gene, while the TrXR1 gene is characterized by high methylation. The Gpx1 gene showed variable methylation; it showed low methylation for a low-fat diet supplemented with or without
EGCG (Figure 2 B).



A



B

Figure 2: Effect of EGCG on DNA methylation levels after diet treatment.

(A) is the mean global DNA methylation levels after diet treatment, and (B) is the DNA methylation levels after diet treatment for a specific gene. We analyzed the results using a one-way ANOVA and expressed the results as the mean + SD. We used Turkey's multiple comparisons test to compare the control group's mean with the treatment group's mean at a 95% confidence level (LFD vs. LFD + EGCG, ND vs. ND + EGCG, and HFD vs. HFD + EGCG for diets). Bars with the letter a were statistically significant in the control group, whereas b was not statistically significant in the control group ($p < 0.05$).

3.3 Effect of EGCG On DNA Methylation Level After Exercise Treatment in Mice

Exercise treatments that include or exclude EGCG correlate with the amount of DNA methylation for four genes (Figure 3 A & B). These genes are Sod2, Gpx1, Cat, and TrxR1. The p-value (0.0001) is less than 0.05, which means that the results are statistically significant. They show that exercise supplementation with or without EGCG affects the DNA methylation levels of the mouse genes Sod2, Gpx1, Cat, and TrxR1. These same results show that a sedentary lifestyle increases DNA methylation (hypermethylation) at the level of genes involved in antioxidant defense. Supplementing EGCG results in a decrease in the level of methylation, also known as hypomethylation. The results showed a statistically significant correlation between the level of DNA methylation in mice and various exercise treatments, either with or without EGCG. p-value = 0.0001. SL supplemented with EGCG showed less methylation (hypomethylation), while SL without EGCG showed high DNA methylation (hypermethylation).

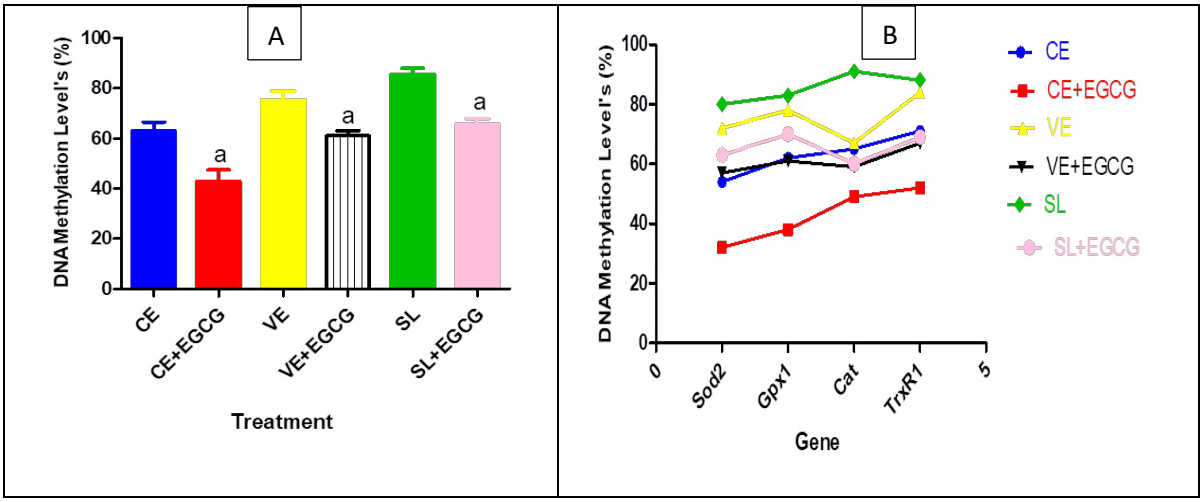


Figure 3: Effect of EGCG on DNA methylation levels after exercise treatment.

(A) shows the mean global DNA methylation levels after exercise treatment, and (B) shows the DNA methylation percentage levels exposed to exercise treatment for a specific gene. We analyzed the data using a one-way ANOVA and expressed the results as the mean. We used Turkey's multiple comparisons test to compare the control group's mean with the treatment group's mean at a 95% confidence level (CE vs. CE + EGCG, VE vs. VE + EGCG, and SL vs. SL + EGCG for exercise). Bars with the letter a were statistically significant in the control group, whereas b was not statistically significant in the control group ($p < 0.05$).

4. Discussion

Exposure to risk factors such as physical inactivity, nutritional fatty acids, dyslipidemia, smoking, hypertension, and diabetes directly correlates with a higher incidence of CVD [25]. These extracellular stimuli cause molecular changes in the DNA, leading to functional and phenotypic changes.

The goal was to test the hypothesis that DNA methylation induced the chronic changes in antioxidant enzyme expression caused by a high-fat diet and a sedentary lifestyle, and to determine if EGCG could reverse these aberrant changes. Researchers have also considered the potential role of EGCG's epigenetic effects on hepatic DNA methylation in explaining its health properties. This is due to the belief that the methylation of DNA plays a crucial role in understanding the negative impact of environmental factors on heart and liver health.

According to Byun et al. DNA methylation is an epigenetic mark that controls gene expression and, by extension, many biological processes, such as embryonic development, gene imprinting, and getting older [26]. Furthermore, the same authors noted that various common diseases like lung cancer, cardiovascular disease, and metabolic disorders are associated with aberrant DNA methylation patterns [26].

In our study, we examined the various mechanisms by which antioxidant compounds could restore DNA methylation patterns altered by chronic diseases. We discovered that the antioxidant EGCG remodels DNA methylation patterns by regulating epigenetic antioxidant enzymes. An HFD without EGCG supplementation exhibited a higher level of DNA methylation. The hypermethylation of The DNA hypermethylation that was seen during an HFD suggests that the mice were severely exposed to both endogenous and exogenous dyslipidemia, which caused Sod2, Gpx1, Cat, and TrxR1 expression to drop. Other studies supported our results. Zhang et al. [27] showed that diet also affects the methylation profile in adults.

Our study also converges with other studies. Mathers et al. showed that nutrients and other dietary components can alter DNA methylation patterns [28] to modulate biological functions that influence health and aging. According to Pirouzpanah et al. higher levels of several micronutrients, such as folate, choline, betaine, methionine, vitamin B6, and vitamin B12, are involved in single-carbon metabolism. These micronutrients contribute to the production of SAM and are associated with increased DNA methylation [29]. In 2006, Fang et al., found that flavonols and (-)-epigallocatechin-3-gallate (EGCG) are competitive inhibitors of DNMTs. They do this by binding to the enzyme's active site [30], which reduces the overall amount of methylation in DNA. Many studies support the hypothesis that food compounds can alter DNA methylation patterns and influence the aging process [31–35].

For exercises, our results showed that SL increases DNA methylation (hypermethylation). Endogenous and exogenous dyslipidemia expose sedentary mice to hypermethylated DNA. This makes Sod2, Gpx1, Cat, and TrxR1 less active. Our study suggests that physical exercise exposes cells to a variety of physical, hormonal, and chemical stimuli, which can alter the expression of

certain genes. Other studies support this hypothesis by suggesting that DNA methylation may play a role in the aforementioned changes, whereas other authors have found results that differ from ours. In a study by Zhang et al. [36], individuals between the ages of 45 and 75 who exercised 26–30 minutes per day had higher overall blood methylation levels compared to those who exercised less than 10 minutes per day [36]. A group of researchers studied the epigenetic response of skeletal muscle following a single exercise session [37]. They observed that after an exercise session, the rate of methylation at the level of the candidate gene promoter decreases, but at kinetics varying from one gene to another [37].

Additionally, for exercise, DNA methylation change was detectable regardless of chronic exercise, voluntary exercise, and sedentary lifestyle compared to these same types of exercise supplemented with EGCG. Sod2 displayed a low level of DNA methylation, followed by Gpx1, TrxR1, and finally Cat. Therefore, the results of this study are of interest, demonstrating that both the high-fat diet and exercise respond to the expression of Gpx1, Cat, and TrxR1 and detect methylation changes. This finding is consistent with various studies that show DNA methylation changes in response to exercise for various skeletal muscle genes [38–40]. In addition to epigenetics, other mechanisms may modulate these changes in Gpx1Cat and TrxR1 expression in response to exercise training. Barres et al. (2012) looked at human skeletal muscle and found that acute exercise causes short-term changes in gene methylation [37]. Expression changes don't happen until after the methylation changes have returned to the levels they were before the exercise [37].

Regarding the methylation of CpG islands, our results showed that an HFD supplemented or not with EGCG had hypermethylation of CpG islands. Similarly, our results showed that an SL supplemented or not with EGCG had hypermethylation of CpG islands. Other studies support our results. Many studies, including those by Laker et al. [38]; Ronn & Ling [39] have shown that EGCG has a big impact on epigenetic regulation events such as histone acetylation, methylation, and DNA methylation. In 2017, Pan et al. discovered that EGCG could stop HDAC1 from working to increase cTnI levels in old mice and make their hearts work better. This shows the complex ways in which EGCG affects epigenetic changes [40].

Diets containing EGCG showed DNA hypomethylation for Sod2, Gpx1, Cat, and TrxR1. This meant that the expression of these genes, which code for antioxidant enzymes, went up, indicating that the body's antioxidant defenses got stronger. Our studies have demonstrated that EGCG reduces DNA methylation in genes involved in antioxidant defense. This means that EGCG increases the expression of genes that code for these enzymes and using EGCG increases the body's antioxidant defense. In the unhealthy setting of CVD, our results show that EGCG has the potential to help prevent or lower cardiovascular risk factors. This is because it increases the body's natural antioxidant defense.

This study demonstrated the ability of antioxidant chemicals to reverse aberrant DNA methylation patterns in chronic illnesses through epigenetic processes. The study revealed that EGCG regulates epigenetic antioxidant enzymes to remodel DNA methylation patterns. Mice fed HFD without EGCG supplementation exhibited a higher level of DNA methylation. The DNA hypermethylation that was seen during an HFD suggests that the mice were severely exposed to both endogenous and exogenous dyslipidemia, which caused Sod2, Gpx1, Cat, and TrxR1 expression to drop. This decrease in endogenous antioxidant defense reflects the onset of a pathological state that can cause CVD over time. Conversely, hypomethylation of the gene encoding the antioxidant enzyme increases its expression, thereby enhancing endogenous antioxidant defense. Muka et al. (2016) observed and concurred with this study, demonstrating that diet also influences the methylation profile in adults [14]. According to Albanese et al. methylation in adult rat livers following an HFD showed variations in both hypomethylation and hypermethylation [41]. In CVD, DNA methylation of CpG sites revealed an inverse relationship with CVD risk [14, 42].

For diets and exercises, it was revealed that HFD and SL increase DNA methylation (hypermethylation). EGCG, whether added or not, decreased the DNA methylation profile for both HFD and SL treatments. Regarding the methylation of CpG islands, our results showed that an HFD supplemented or not with EGCG had hypermethylation of CpG islands. Similarly, our results showed that an SL supplemented or not with EGCG had VE and a CE supplemented or not with EGCG. These findings imply that certain polyphenols may target particular organs and activities [43]. For example, these findings on DNA methylation after food and exercise raise questions about our understanding of the dietary modulation of DNA methylation processes. Nutrition can alter DNA methylation patterns in a variety of ways, according to the answer. Other writers have agreed with this theory, whereby rodents with diets low in methyl donors have hypomethylated global DNA [44]. Importantly, this is a time when individuals are particularly susceptible to environmental factors, such as nutrition, which might impede the proper creation of very persistent epigenetic markers [45]. That is why nutritional problems at a young age of development can have long-term implications, according to Mozhui et al. [46]. There is further evidence that maternal diet influences epigenetic signatures in children. Similarly, Yu et al. said that maternal HFD caused worldwide DNA hypermethylation, including fatty acid, cholesterol metabolism, and lactation genes [47]. Researchers examined DNA methylation in exercise and diet plans that included EGCG and found that the use of EGCG lowered the DNA methylation profile (hypomethylation), while treatments without EGCG raised the DNA methylation profile (hypermethylation).

5. Conclusion

The study investigated DNA methylation changes in the livers of mice at risk for cardiovascular disease (CVD). The study revealed

that the antioxidant genes were over-methylated in the liver due to a high-fat diet (HFD) and sedentary lifestyle (SL). However, supplementation with EGCG decreased DNA methylation, indicating EGCG's role in causing DNA hypomethylation. This suggests that a high-fat diet and sedentary lifestyle induce dyslipidemia, impairing antioxidant defense mechanisms partly through epigenetic mechanisms. The study shows that bioactive antioxidants might be able to change the epigenome and fix DNA methylation patterns that aren't working right, which would lower oxidative stress. We suggest further research on the impact of diet on DNA methylation in humans, despite the challenges posed by ethical considerations. Understanding these processes could lead to the strategic use of antioxidant substances and DNA methylation-modifying medicines to prevent and treat chronic diseases like CVD. We need more comprehensive research to determine the optimal dosage and duration of EGCG supplementation for CVD patients.

Declaration

In research, there is no competing interest as the research has been experimental on laboratory animal models (mice).

Ethics Approval

The research has been approved by the Animal Ethics Committee of Kampala International University and the Uganda National Council of Science and Technology; the Approval number is NS 645.

Consent to Publish

Before submitting the manuscript, there was consent of the authors for its submission

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