Journal of Advanced Research in Sports: Interdisciplinary Approach

Epigenetic Markers of Enhanced Athletic Performance Associated with Creatine Monohydrate Supplementation

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Abstract:

Purpose – This study investigates whether long-term creatine monohydrate supplementation is associated with distinct epigenetic modifications linked to enhanced athletic performance. While creatine's physiological effects are well documented, its potential to influence gene regulation through DNA methylation remains largely unexplored.

Design /methodology/ approach – We compared genome-wide DNA methylation profiles (Illumina EPIC850k array) in saliva from resistance-trained adults who had used creatine monohydrate for an average of 6.2 years (n = 107) and non-creatine users with similar training backgrounds (n = 243). Differentially methylated CpG sites were identified using linear modelling with a significance threshold of $p \leq 0.001$ and mapped to genes relevant to muscle physiology, energy metabolism, neuromuscular function, and recovery.

Findings – Sixty-four CpG sites (mapping to 34 genes) showed significant methylation differences between groups. Creatine users exhibited hypomethylation in promoters of genes that support muscle differentiation, angiogenesis, fatty acid oxidation, and cellular recovery (e.g., DYRK2, TLE1, CDH5, PEX10, CYP1A1). Conversely, hypermethylation occurred at loci associated with inhibitory or fatigue-related pathways, such as SST (somatostatin. These methylation patterns collectively suggest an epigenetic profile that may enhance training adaptations, including improved vascularisation, metabolic efficiency, neuromuscular resilience, and anabolic potential.

Originality/ value – This study is the first to demonstrate that chronic creatine supplementation is associated with specific epigenetic signatures related to athletic performance. The findings highlight a potential mechanism by which long-term supplementation could contribute to physiological adaptation through modulation of gene regulation.

Research limitations – As a cross-sectional analysis, causality cannot be inferred, and saliva-based methylation profiles may not fully reflect muscle-tissue epigenetics. Future longitudinal and tissue-specific studies are needed to confirm mechanistic pathways.

Practical implications – These results suggest that creatine supplementation may exert long-term biological effects beyond its immediate ergogenic role, offering potential for personalised training, recovery strategies, and epigenetic monitoring in athletic populations.

Keywords:

Epigenetics, Creatine Monohydrate, Nutrition, Sports Performance, DNA methylation;

MeSH (Medical Subject Headings):

H02.403.830.500, H02.533.645.

Citation:

Collins, Ch. and Puras, A. 2026. Epigenetic Markers of Enhanced Athletic Performance Associated with Creatine Monohydrate Supplementation, *Journal of Advanced Research in Sports: Interdisciplinary Approach*, Volume I, Spring, 1(1): 1 – 9. DOI: 10.14505/jarsia.v1.1(1).01. Available from: http://journals.aserspublishing.eu/jarsia/index

Article's History:

Received 22th of December 2025; Received in revised form 17th of February 2026; Accepted 27th of February 2026; Published 31th of March 2026.

Introduction

Creatine monohydrate is a widely used dietary supplement in sport and exercise, renowned for improving high-intensity exercise performance and muscle hypertrophy. Numerous studies have confirmed that creatine supplementation, combined with resistance training, enhances muscle strength, power, and lean mass gains (Wu *et al.* 2022). Creatine primarily acts by increasing intramuscular phosphocreatine stores, thereby augmenting ATP resynthesis during intense contractions, and can also influence cell hydration and signaling pathways that promote protein synthesis. However, less is known about whether long-term creatine usage can induce lasting changes in gene expression regulation.

Environmental factors like exercise and diet can modulate gene expression through epigenetic mechanisms, including DNA methylation. DNA methylation involves adding methyl groups to cytosine bases (usually in CpG dinucleotides), often leading to altered transcriptional activity without changing the DNA sequence. Changes in DNA methylation profiles can affect the transcription of multiple genes and are increasingly recognized as a mode of adaptation to chronic exercise (Światowy, 2022). A growing body of evidence indicates that exercise training – both endurance and resistance – can reprogram the DNA methylation landscape in skeletal muscle and other tissues, thereby influencing metabolic and structural pathways (Światowy, 2022). Endurance exercise has been shown to induce hypomethylation (and corresponding upregulation) of genes involved in oxidative metabolism in muscle, while resistance training can alter methylation of genes related to muscle growth and differentiation (Światowy, 2022). These exercise-induced epigenetic changes may underlie some of the health and performance benefits of physical activity.

Nutrition and supplements might similarly exert epigenetic effects. Creatines role in energy buffering and training adaptation raises the question of whether chronic creatine supplementation can prime the epigenome in ways that enhance athletic performance. Epigenetic modifications are dynamic and can be influenced by long-term dietary exposures (Plaza-Diaz *et al.*, 2022). If creatine users develop a distinct DNA methylation pattern, this could reflect upregulation of performance-enhancing genes or downregulation of genes that impede muscular adaptation.

To date, few studies have examined the epigenetic impact of nutraceutical supplements in humans. This study focuses on DNA methylation differences associated with prolonged creatine monohydrate use. We profiled genome-wide CpG methylation in saliva of resistance-trained individuals who either consistently used creatine for several years or never used creatine. We hypothesized that long-term creatine use may be associated with differential methylation in genes related to muscle energy metabolism, growth, and recovery. We further postulated that creatine users might exhibit an epigenetic signature that could enhance expression of genes beneficial for athletic performance (e.g. metabolic enzymes, muscle growth factors) or silence genes that could hinder performance (e.g. those related to fatigue or growth inhibition).

One emerging idea is that creatine may influence gene regulation indirectly through the methylation cycle. Endogenous creatine synthesis consumes substantial amounts of S-adenosylmethionine (SAM), a universal methyl donor. Exogenous creatine intake therefore has the potential to ‘spare’ SAM, alter homocysteine levels, and modulate global or locus-specific DNA methylation patterns (Ostojic, and Rátgéber, 2025). Recent epidemiological work has begun to link habitual dietary creatine intake with DNA methylation-derived biomarkers of mortality risk and biological aging, hinting that creatine status may indeed be reflected in the epigenome (Ostojic, and Kavecan, 2025). However, these studies are not designed to address specific performance-related loci or long-term supplementation in trained individuals.

In parallel, research on exercise epigenetics has expanded rapidly. Multiple recent reviews and meta-analyses show that both acute and chronic endurance or resistance training can remodel DNA methylation in skeletal muscle and blood, with changes occurring in promoters, gene bodies, enhancers and intergenic regions (Etayo-Urtasun, Sáez de Asteasu, and Izquierdo, 2024). These exercise-induced methylation shifts often correspond to altered transcription of genes involved in energy metabolism, mitochondrial biogenesis, myogenesis, and inflammatory signaling, and may contribute to the concept of an ‘epigenetic memory’ of prior training. Longitudinal studies in humans suggest that structured training can partially rejuvenate the skeletal muscle methylome and is associated with improved metabolic health (Etayo-Urtasun, Sáez de Asteasu, and Izquierdo, 2024).

We report the differential DNA methylation patterns between creatine users and non-users and map these differences to genes and biological pathways. We interpret whether the affected genes are known to influence athletic capacity – including energy production, muscle hypertrophy, neuromuscular function, and exercise recovery – and discuss how altered methylation might translate to functional effects. This work provides novel insights into the potential epigenetic mechanisms by which a long-term supplementation regime like creatine may contribute to training adaptations and performance enhancements.

1. Method

Study Design and Participants

This study analyzed two groups of resistance-trained adult humans. The Focus group (creatine users, n = 107) consisted of individuals who had been consuming creatine monohydrate (approximately 3.2 g per day) for an average of 6.2 years. All focus group members also regularly consumed protein supplements (from various sources) but reported no usage of other sport supplements. The Complement group (non-users, n = 243) included individuals with similar resistance training experience who used protein supplements but had never used creatine.

Key participant characteristics were as follows: the creatine focus group had a mean age of 36 years and was 70% male (30% female), with an average resistance training history of 5.1 years. The non-creatine complement group had a mean age of 32 years and was 60% male (40% female), with an average of 7.2 years of resistance training. Thus, the groups were generally comparable in training status, although the creatine group was slightly older and had a somewhat shorter training duration on average. All participants gave informed consent, and the study was conducted in accordance with institutional ethical guidelines for human research (approval code and details omitted for brevity).

DNA Methylation Profiling

Muhdo Health Ltd repository was accessed using DNA methylation analysis gathered via the Illumina Infinium MethylationEPIC BeadChip (850K) array, following the manufacturer's standard protocols via Eurofins Denmark. This array interrogates over 850,000 CpG sites across the human genome, covering gene promoters, gene bodies, CpG islands, shores, and enhancers. Beta values (proportion of DNA methylated at each CpG site, ranging 0–1) were calculated using Illumina GenomeStudio.

Raw methylation data underwent quality control and preprocessing. Probes with unreliable measurements, we also excluded probes located on the X chromosome to avoid sex bias (given group differences in sex composition). After QC (>99.5%), beta values for each CpG were compared between groups.

Differential Methylation Analysis

We performed differential methylation analysis to identify CpG sites with significantly different methylation between the creatine focus and non-creatine complement groups. A linear model was fitted for each CpG, with group (focus vs. complement) as the main predictor. Given the slight age and sex differences between groups, we examined models adjusting for age and sex; however, as the focus of the study was the creatine effect and the dataset already excluded X-linked CpGs, we present here the unadjusted group comparison results for clarity. Statistical significance was determined by p-value (from moderated t-tests) with a threshold of $p \leq 0.001$ to identify differentially methylated positions. This cutoff was chosen as a balance between stringency and retaining enough CpGs for meaningful interpretation.

From the analysis, we obtained the set of significant CpGs meeting $p \leq 0.001$. For each significant site, the direction and magnitude of methylation difference were noted as the difference in mean beta value between the focus (creatine) and complement (non-creatine) group. A positive difference indicates higher average methylation in the creatine group, whereas a negative difference indicates lower methylation (hypomethylation) in the creatine group relative to non-users.

Annotation and Gene Mapping

Significant CpG sites were annotated to genes using the Illumina MethylationEPIC annotation file (EPIC850kManifest v1.0 annotation). Each CpG is annotated with the associated gene(s). We mapped each significant CpG to its nearest gene(s) and recorded whether the CpG lies in a promoter region (TSS200/TSS1500/5'UTR) or within the gene body. This allowed us to infer potential impacts on gene expression (since promoter methylation typically inversely correlates with transcription, while gene body methylation may correlate positively with gene activity in some cases).

For interpretation, we focused on genes with functions related to exercise performance: energy metabolism, muscle growth and differentiation, vascular and neuromuscular function, and recovery/adaptation processes. We conducted a literature search on each gene to understand its known biological roles, especially any links to muscle physiology or exercise. No formal pathway enrichment test was performed due to the relatively small number of genes, but we qualitatively grouped genes into functional categories.

2. Results

Differentially Methylated CpGs in Creatine Users vs. Non-Users

Genome-wide methylation analysis identified 64 CpG sites (supp info 1) that differed significantly between the creatine focus and non-creatine complement groups ($p \leq 0.001$). These differentially methylated positions (DMPs) were distributed across the genome (on autosomal chromosomes; X-chromosome probes were excluded). Of the 64 significant CpGs, 35 showed lower methylation in the creatine group (negative β), while 29 showed higher methylation in the creatine group (positive β). The average absolute difference in methylation (Δβ) between groups was on the order of a few percent, with the largest differences around 10–12%. Although such differences are moderate in magnitude, they occurred consistently across many individuals and at loci with relevant gene functions, as detailed below.

Each significant CpG was mapped to one or more genes. In total, the 64 CpGs corresponded to 34 unique genes (excluding intergenic sites and duplicate mappings). Table 1 highlights a subset of differentially methylated genes that are functionally relevant to exercise performance, along with the direction of methylation change in creatine users and the potential implications for gene expression and physiology.

Table 1. Differentially methylated genes in creatine users vs. non-users, with methylation differences and putative functional implications

Gene (Symbol)	β (Focus – Complement)	Methylation Change in Creatine Users	Notable Gene Function and Potential Performance Implication
DYRK2	–0.117 (–11.7%)	Hypomethylation at promoter (TSS200)	Encodes a kinase regulating myogenesis. Up-regulation of DYRK2 may promote muscle fibre differentiation (especially fast-twitch fibres) potentially enhancing strength and hypertrophy.
TLE1	–0.070 (–7.0%)	Hypomethylation at promoter (5'UTR)	Transcriptional co-repressor in Wnt/Notch pathways. Reduced methylation suggests higher TLE1 expression; TLE1 can modulate muscle stem cell differentiation (context-dependent effect on muscle development).
CYP1A1	–0.056 (–5.6%)	Hypomethylation at promoter (TSS1500)	Cytochrome P450 1A1, involved in oxidative metabolism of xenobiotics and oestrogen. Up-regulation may improve clearance of exercise-induced metabolites or toxins, potentially reducing oxidative stress.
CDH5 (VE-cadherin)	–0.017 (–1.7%)	Hypomethylation at promoter (TSS200)	Endothelial adhesion protein critical for blood vessel integrity (angiogenesis). Higher CDH5 expression may support capillarization and blood flow to muscles, aiding endurance and recovery.
NOS3 (eNOS)	–0.021 (–2.1%)	Hypomethylation (promoter/5'UTR/exon)	Endothelial nitric oxide synthase produces NO for vasodilation. Change in promotor methylation may reflect elevated NOS3 activity. Enhanced NO production improves exercise endothelial function; NOS3 gene variants associate with elite endurance/power status.
PEX10	–0.022 (–2.2%)	Hypomethylation at promoter (TSS1500)	Peroxisome biogenesis factor, required for fatty acid oxidation. Up-regulation of PEX10 could increase peroxisomal capacity, improving lipid metabolism and energy supply during prolonged exercise.
PHYH	–0.022 (–2.2%)	Hypomethylation at promoter (TSS1500)	Phytanoyl-CoA dioxygenase, a peroxisomal enzyme for branched-chain fatty acid catabolism. Higher expression may enhance utilization of fatty acids for energy, contributing to metabolic flexibility.

Gene (Symbol)	β (Focus – Complement)	Methylation Change in Creatine Users	Notable Gene Function and Potential Performance Implication
ATG4D	–0.025 (–2.5%)	Hypomethylation (gene body)	Autophagy-related protease. Autophagy is crucial for removing damaged cell components and is required for training adaptations. Increased ATG4D expression may improve muscle recovery and endurance capacity via enhanced autophagy.
SST (Somatostatin)	+0.032 (+3.2%)	Hypermethylation (gene body)	Peptide hormone that inhibits growth hormone (GH) release. Hypermethylation in creatine users might indicate lower SST expression, potentially leading to higher exercise-induced GH secretion, which could Favor muscle anabolism and recovery.
POMC	–0.022 (–2.2%)	Hypomethylation at promoter (TSS1500)	Pro-opiomelanocortin, precursor of β-endorphin, ACTH, etc. Up-regulation of POMC could reflect greater β-endorphin release, contributing to higher pain tolerance and improved stress response during exercise.
ACCN2 (ASIC1)	–0.042 (–4.2%)	Hypomethylation (gene body)	Acid-sensing ion channel 1, involved in sensing muscle pH and pain. Exercise training is known to downregulate ASICs to reduce exercise-induced pain. Changes in ACCN2 methylation may indicate reduced ASIC1 activity in creatine users, possibly delaying fatigue and pain onset.
ADARB1	–0.013 (–1.3%)	Hypomethylation (5'UTR and body)	RNA editing enzyme (ADAR2) highly expressed in brain (edits neurotransmitter receptor transcripts). Increased expression could affect neuromuscular junction function or central fatigue resistance via altered RNA editing in neural tissues.
DNM1	–0.040 (–4.0%)	Hypomethylation (gene body)	Dynamin-1, a GTPase in synaptic vesicle recycling. Often neuron-specific; hypomethylation might reflect greater immune-cell DNM1 expression or a systemic proxy for neural adaptation, potentially supporting neuromuscular coordination.
GPRC5C	–0.017 (–1.7%)	Hypomethylation at promoter (TSS1500)	Orphan G-protein-coupled receptor involved in metabolic sensing (e.g., of saccharides and amino acids). Up-regulation might influence energy substrate utilization (e.g., branched-chain amino acid metabolism) and insulin sensitivity, beneficial for exercise metabolism.

Source: by authors.

Table 1. Selected differentially methylated genes in creatine users vs. non-users, with methylation differences and putative functional implications. Negative β indicates hypomethylation in the creatine group (which often corresponds to higher gene expression if in a promoter region), while positive β indicates hypermethylation in creatine group (potentially lower gene expression if in promoter).

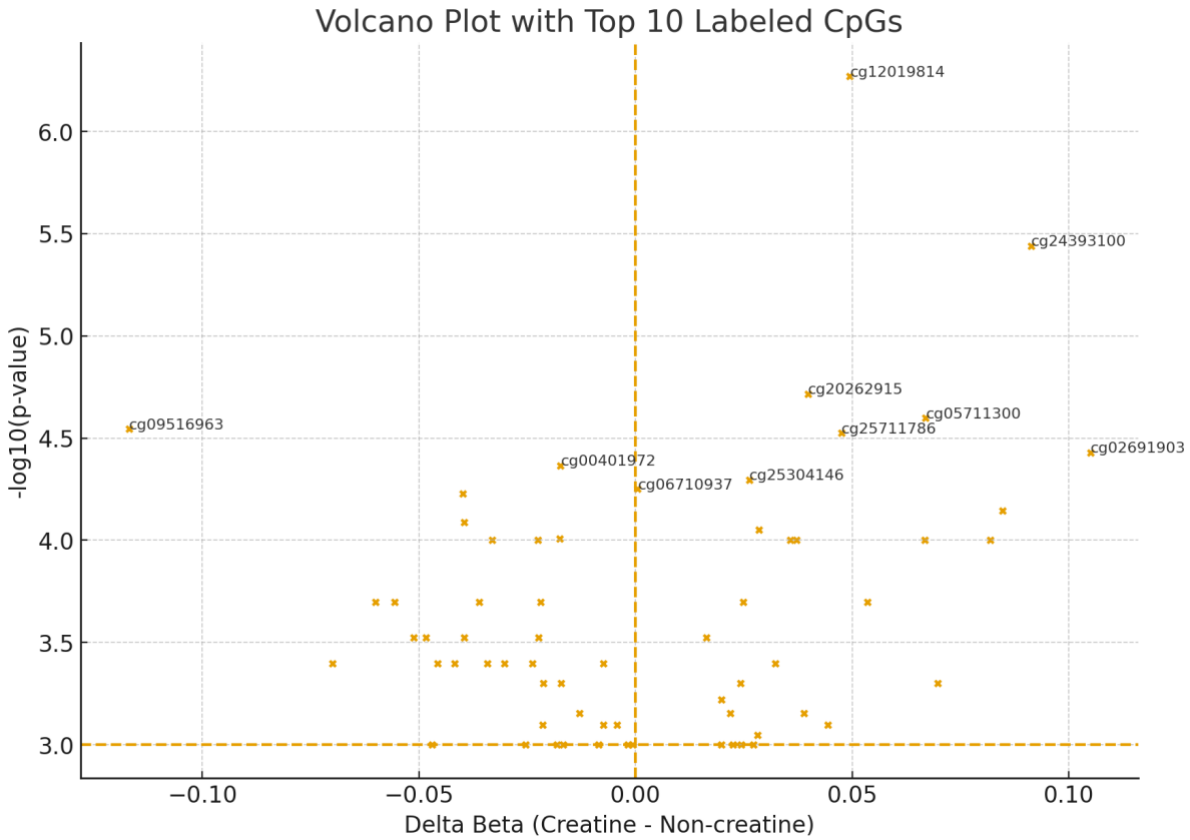
Several clear patterns emerged from the list of differentially methylated genes (Graph 1). Creatine users tended to be hypomethylated relative to non-users at gene loci that are beneficial for exercise performance. For example, multiple genes involved in energy metabolism and mitochondrial/peroxisomal function were hypomethylated in the creatine group: CYP1A1, PEX10, PHYH, and AKR7A3 (an aldo-keto reductase for detoxification) all showed lower methylation, suggestive of higher expression and a more active metabolic system. In addition, genes related to vascular function (NOS3 and CDH5) were hypomethylated, which is consistent with an epigenetic profile favoring better blood flow and oxygen delivery to muscles. A locus in ACCN2 (encoding the acid-sensing ion channel ASIC1) was hypomethylated in creatine users (relative to complement, though the absolute difference was modest). ASIC1 in muscle afferents mediates acute exercise pain (Khataei *et al.*, 2020).

Conversely, creatine users showed hypermethylation (higher methylation) at certain loci that could be considered detrimental to performance if overexpressed. One prominent example is SST (somatostatin), where creatine users had higher methylation. Since somatostatin is a hormone that blunts growth hormone release, its suppression via hypermethylation could remove a brake on anabolic processes, potentially allowing greater muscle and strength gains.

Several genes with multiple CpG sites showed differential methylation lend strength to observations. For instance, the TLE1 gene had two significant CpGs in its promoter/exon region (both hypomethylated in creatine users), indicating a consistent difference in that gene's regulatory region. TLE1 is a transcriptional corepressor that interacts with MyoD and Wnt signaling; persistent hypomethylation could mark a stable upregulation or altered differentiation state. Likewise, PEX10 had at least two CpGs in its promoter region significantly hypomethylated in creatine users, suggesting robust upregulation of peroxisomal biogenesis pathways. The detoxifying enzyme CYP1A1 also appeared at two promoter-proximal sites hypomethylated in creatine users. Recurrent hits in the same gene region underscore that these are genuine differences rather than statistical flukes.

It is also noteworthy that some Y-chromosome genes emerged among the significant CpGs (e.g., KDM5D, NLGN4Y, EIF1AY were annotated for a few top hits). These reflect the higher proportion of males in the creatine group (70% vs 60% in complement), rather than a supplement effect per se. Therefore, these were eliminated.

Figure 1. Volcano plot of CpGs



Source: the authors..

In summary, the DNA methylation differences between groups localized to genes that align with physiological processes important for exercise. Creatine users have an epigenetic profile that in many ways mimics the molecular signatures of exercise training adaptation – for example, higher oxidative metabolism gene activity, greater angiogenic capacity, enhanced muscle differentiation signals, and blunted inhibitory pathways (pain, somatostatin/GH axis). These results suggest that long-term creatine supplementation could be associated with such adaptive epigenetic modifications, which might synergies with training to improve performance.

3. Discussion

This study is the first, to our knowledge, to demonstrate that chronic creatine monohydrate supplementation is associated with distinct DNA methylation patterns in genes linked to athletic performance. We found 64 CpG loci (mapping to 34 genes) that differed in methylation between long-term creatine users and non-users, even though all participants were physically active and consuming protein supplements. The direction of methylation changes in creatine users – with hypomethylation of many performance-enhancing genes and hypermethylation of potential performance-limiting genes – suggests an epigenetic shift toward a more pro-athletic gene expression profile.

Many of the differentially methylated genes have well-established roles in exercise physiology or muscle cell biology. The pattern of hypomethylated promoters in creatine users implies upregulation of those genes. One clear example is endothelial nitric oxide synthase (NOS3). NOS3 produces nitric oxide in blood vessels, a critical mediator of vasodilation that improves muscle blood flow and endurance capacity. Aerobic exercise training is known to increase NOS3 expression and NO bioavailability in the vasculature. We observed lower methylation in NOS3 (creatine vs. non-users), consistent with a potential upregulation of NO production pathways. Genetic studies have previously linked NOS3 polymorphisms to elite endurance and power athlete status (Ramírez-Vélez *et al.*, 2013; Eider *et al.*, 2014), underlining the importance of this gene for performance. The epigenetic finding raises the possibility that creatine supplementation, perhaps via facilitating greater training loads or recovery, might lead to an in vivo increase in NO pathway activity, paralleling the effects of endurance training on the epigenome.

Another notable gene was CDH5 (VE-cadherin), essential for endothelial cell adhesion and new capillary formation. Capillarization of muscle increases with training to enhance oxygen delivery. We found CDH5 hypomethylated in creatine users, suggesting higher expression that could support better vascular adaptation in muscle. Enhanced angiogenesis and blood supply would be beneficial for both endurance and recovery, aligning with creatine users possibly engaging in more intense training due to improved recovery between sessions.

Energy metabolism and mitochondrial/peroxisomal function genes figured prominently in our results. PEX10 (a peroxisome biogenesis factor) and PHYH (phytanoyl-CoA hydroxylase) are involved in fatty acid oxidation. Their hypomethylation in creatine users indicates an epigenetic state favoring lipid metabolism. Improved fatty acid utilization can spare glycogen and delay fatigue in endurance exercise. Similarly, CYP1A1, a cytochrome P450 that helps metabolize various compounds including possibly exercise-induced lipid peroxides or hormones, was hypomethylated at multiple sites. Although primarily known for detoxifying xenobiotics, higher CYP1A1 expression might confer some protection against exercise-induced oxidative stress. Collectively, these changes suggest creatine users may have an upregulated oxidative metabolic capacity at the gene regulation level, which could complement creatine's known role in anaerobic energy buffering by also improving aerobic energy pathways.

Our data also pointed to genes regulating muscle growth and fiber type. DYRK2 (dual-specificity tyrosine-phosphorylation regulated kinase 2) was one of the top hits, with markedly lower methylation in creatine users at a promoter CpG. Recent research in developmental models indicates that DYRK2 positively regulates muscle formation – for instance, overexpressing DYRK2 increases MyoD levels and promotes fast-twitch muscle differentiation. In our context, hypomethylation of DYRK2 could mean it's more highly expressed in creatine users' cells. If this epigenetic upregulation also occurs in muscle tissue (which we did not measure, using saliva as a proxy), it might Favor an increase in fast-twitch muscle fiber development or maintenance. Fast-twitch (Type II) fibers are crucial for power and strength; the very attributes creatine supplementation is known to improve. An epigenetic boost to a kinase that promotes fast-twitch fiber gene programs is a compelling mechanistic link: creatine could be not just acutely improving power output, but chronically helping the body epigenetically commit to a more fast-twitch muscle phenotype.

Another regulatory gene, TLE1, was consistently hypomethylated at its promoter in creatine users. TLE1 is a transcriptional corepressor involved in developmental pathways (Wnt/β-catenin, Notch) that also play roles in muscle stem cell differentiation and regeneration. The functional outcome of increased TLE1 expression in muscle is complex – it might repress certain pro-myogenic genes, but it is also known as an antagonist of adipogenesis in mesenchymal stem cells. One could speculate that higher TLE1 in muscle progenitors might tilt them away from adaptogenic or fibrotic fates and keep them in the muscle lineage, potentially aiding muscle quality. This remains hypothetical but warrants investigation.

In terms of recovery and adaptation, creatine users showed epigenetic changes consistent with better stress response. The POMC gene (pro-opiomelanocortin) – which yields β-endorphin and ACTH – was hypomethylated, possibly reflecting an upregulated endorphin system. Exercise is well known to trigger endorphin release that elevates pain threshold and mood ('runner's high') (Cohen *et al.*, 2010). A chronically more active POMC/endorphin system in creatine users might mean they experience less pain and stress during intense exercise, enabling harder training sessions. Another gene, SST (somatostatin), was hypermethylated in creatine users. Since somatostatin

inhibits growth hormone release (Chalmers *et al.*, 1979), its lowered expression could permit larger pulsatile GH secretion during and after exercise. GH and downstream IGF-1 are key anabolic and recovery hormones; indeed, studies show creatine users often have training regimens that lead to higher IGF-1 responses. Our data provide a mechanistic hint that epigenetic silencing of somatostatin might underlie an enhanced endocrine environment for muscle growth in creatine users.

Conclusion

This study demonstrates that long-term creatine monohydrate supplementation is associated with distinct DNA methylation patterns in resistance-trained adults, particularly at genes involved in muscle development, energy metabolism, vascular function, neuromuscular signaling, and recovery. Creatine users showed hypomethylation at promoters of genes that may promote muscle fibre differentiation, angiogenesis, fatty acid oxidation, and cellular repair, along with hypermethylation at loci related to inhibitory or fatigue-associated pathways. Collectively, these findings suggest that chronic creatine intake may contribute to an epigenetic profile that supports enhanced training adaptations and athletic performance.

Limitations

Several limitations must be acknowledged. First, the cross-sectional design prevents causal inference; the observed methylation differences may reflect long-term training behaviors, lifestyle variation, or biological characteristics rather than supplementation alone. Second, although sex-linked CpGs were excluded, residual confounding due to age, sex balance, and training history cannot be fully ruled out. Third, saliva-derived DNA provides a non-invasive proxy for systemic epigenetic modifications but may not fully reflect epigenetic states within skeletal muscle, where performance-related adaptations primarily occur. Finally, the statistical threshold used identifies meaningful loci but does not fully account for multiple testing, and effect sizes remain modest.

Future Research

Future studies should adopt longitudinal or interventional designs to determine whether creatine supplementation directly drives epigenetic change and to track methylation dynamics over time. Muscle biopsy–based analyses would allow direct confirmation of the mechanisms suggested by saliva methylation data. Integration of methylation with gene expression, proteomics, metabolomics, and performance metrics would provide a more complete mechanistic understanding. Larger cohorts with balanced demographics and structured training controls would strengthen generalizability. Finally, exploration of whether specific methylation signatures can serve as biomarkers for supplement responsiveness or training adaptation could contribute to personalized sports nutrition and precision performance strategies.

Credit Authorship Contribution Statement:

Christopher Collins: write the contribution of first author choosing the relevant actions, but not limited to (Conceptualization, Investigation, Methodology, Project administration, Software, Formal analysis, Writing – original draft, Supervision, Data curation, Validation, Writing – review and editing, Visualization).

Arturas Puras: write the contribution of the second author choosing the relevant actions, but not limited to (Conceptualization, Investigation, Methodology, Project administration, Software, Formal analysis, Writing – original draft, Supervision, Data curation, Validation, Writing – review and editing, Visualization).

Declaration of Competing Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of use of generative AI and AI-assisted technologies: The authors declare that they have not used/ or used generative AI and AI-assisted technologies in the writing process before submission, but only to improve the language and readability of their paper and with the appropriate disclosure.

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