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Investigating the Role of H3K27me3 Demethylation in VEGFA Gene Activation Following Acute Exercise in Human Skeletal Muscle: A Research Proposal

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Abstract

Skeletal muscle adapts to endurance exercise by tissular remodelling such as angiogenesis, the growth of novel capillaries, a response largely mediated by transcriptional activation of vascular endothelial growth factor A (VEGF). While VEGF expression is classically regulated by the hypoxia-inducible transcription factor (HIF), recent evidence in endothelial cells suggests that epigenetic mechanisms—specifically, the removal of the repressive histone mark H3K27me3—also play a key role in activating this angiogenic gene. It remains unclear however, whether skeletal muscle myocytes utilize similar chromatin-based regulation for VEGF transcription during exercise. This study proposal aims to determine whether acute endurance exercise in humans induces epigenetic remodeling at the VEGFA promoter in skeletal muscle through H3K27me3 demethylation, and whether this is accompanied by upregulation of histone demethylases. Recreationally active male and female participants will complete 60 minutes of treadmill running at 70–80% of their maximal oxygen uptake. Muscle biopsies will be collected from the vastus lateralis at baseline, immediately post-exercise, and 1- and 3-hours post-exercise. Chromatin immunoprecipitation followed by quantitative-PCR (ChIP-qPCR) will be used to assess H3K27me3 enrichment at the VEGFA promoter. VEGFA mRNA expression will be measured via real-time quantitative PCR, and protein expression of the histone demethylases JMJD3 (KDM6B) and KDM4B will be quantified by immunoblotting. Our hypothesized results include a time-dependent decrease in H3K27me3 enrichment at the VEGFA promoter post-exercise, with significant reductions at 1 and 3 hours. This will be paralleled by increased VEGFA mRNA expression and a rapid and sustained upregulation of JMJD3 and KDM4B protein levels. These results would suggest that myocytes may employ a conserved epigenetic mechanism, previously observed in endothelial cells, to regulate VEGFA expression in response to exercise-induced hypoxia. The coordinated reduction in H3K27me3 and upregulation of demethylases supports a chromatin remodeling model of angiogenic gene activation. This study would broaden our understanding of exercise-induced molecular adaptation by implicating histone modifications as regulators of VEGF expression in human skeletal muscle. Future work should investigate the cell-type specificity of these changes and their implications for long-term skeletal muscle remodeling to endurance exercise.

Keywords: exercise; VEGFA; H3K27me3; skeletal muscle; epigenetics; histone demethylases; hypoxia

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Introduction

Skeletal muscle represents one of the largest tissues of the body accounting for roughly 40% of total body mass [1]. The skeletal muscle is a remarkable organ involved in various biological processes such as glucose disposal, thermoregulation and most importantly locomotion and movement [1]. To accommodate the energetic needs of this highly metabolic tissue the skeletal muscles is equipped with an impressive network of capillaries that intercalate between myofibers [2]. Indeed, as structure defines function, these capillaries play a vital role in supporting exercising muscles by facilitating the delivery of oxygen, glucose and free-fatty acids to contracting myofibers [3]. Furthermore, capillaries allow for muscle-organ communication throughout the body via the circulation of myokines [4].

Notably, exercise serves as a powerful physiological stimulus that triggers a range of adaptations in the skeletal muscle tissue [5]. One such adaptation is skeletal muscle angiogenesis, the process of novel capillary formation from pre-existing blood vessels [6]. During angiogenesis endothelial cells, the main cellular components of capillaries, proliferate, migrate, and arrange into vascular tubes leading to the formation of a novel capillary [7]. After skeletal muscle angiogenesis has occurred, the expanded capillary network results in a greater capillary-myofiber surface area, facilitating more efficient oxygen and nutrient delivery and in turn enhanced exercise performance [8].

Among regulators of skeletal muscle angiogenesis, the Vascular Endothelial Growth Factor-A (VEGF) is widely recognised as the master orchestrator [9]. During exercise, VEGF transcription is rapidly increased in myofibers

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particularly due to the oxygen and metabolic demands solicited by repeated contractile activity [10]. Once translated into a functional protein, myofiber-derived VEGF is released into the muscle interstitial space where it binds to receptors on nearby capillary endothelial cells and initiates signaling cascades that drive capillary growth [11]. The essential role of VEGF in skeletal muscle angiogenesis was confirmed by Olfert et al., who found that mice with a myofiber-specific VEGF deletion had significantly reduced capillary density [12]. Furthermore, despite elevated expression of other angiogenic genes and metabolic adaptations, exercise-induced capillary growth was severely impaired, and aerobic exercise capacity was dramatically reduced [12]. These findings demonstrate a non-redundant role of VEGF, and its absence cannot be compensated for by other pro-angiogenic pathways.

Due to the metabolic stress imposed by exercise, contracting myofibers experience a drop in cellular oxygen tension (PO2) leading to transient hypoxic conditions [13]. These hypoxic conditions stabilize the alpha subunit of the Hypoxia Inducible Factor 1 (HIF-1α), transcription factor that is normally produced but rapidly degraded when under normoxia [14]. Once stabilized, HIF-1α enters the nucleus where it binds to a constitutively expressed beta subunit (HIF-1β) leading to transcription of target genes with hypoxia response elements (HRE) promoters, including VEGF [14]. In accordance, Ameln et al. demonstrated exercise to stabilize HIF-1α protein and promote its ability to bind HRE in human skeletal muscle concomitant with increased VEGF transcription [15]. Notably, when blood flow to the exercising leg was deliberately restricted, creating a more pronounced hypoxic environment, HIF-1α activation and VEGF transcription were even further amplified [15].

While HIF-1α clearly plays a major role in regulating VEGF transcription in skeletal muscle during exercise-induced hypoxia, recent evidence suggests that VEGF activation may also rely on changes in chromatin structure. Liu et al. (2020) showed that in endothelial cells HIF-1α directly promotes the expression of histone demethylases such as KDM4B and KDM6B, which respond to hypoxia by removing the H3K27me3 repressive mark from regions near the VEGFA gene [16]. This chromatin remodeling facilitates VEGFA transcription in parallel with HIF-1α's direct action on the VEGFA promoter. While this mechanism is well established in endothelial cells, it remains unclear whether myocytes have a similar epigenetic pathway to regulate VEGFA expression during exercise-induced hypoxia. Our study aims to investigate whether these epigenetic mechanisms are conserved in myocytes. To address this aim, we propose a human acute exercise protocol in which vastus lateralis biopsy samples will be assessed for H3K27me3 enrichment on the VEGFA promoter via ChIP-qPCR in combination with VEGFA gene expression analysis via RT-qPCR (Figure 1).

Methods

Subject Criteria

Healthy, recreationally active male and female participants aged 20–30 will be recruited. Individuals will be excluded if they are currently engaged in structured endurance or resistance training, have a history of cardiovascular, metabolic, or musculoskeletal disorders, smoke, or are taking medications that might affect vascular or gene expression responses. Physical activity level will be assessed using a standardized questionnaire to ensure participants meet the criteria of being generally active but not trained. A PARQ questionnaire will be used to determine if individuals are fit for exercise.

Acute Exercise Protocol and Biopsy Sampling

To determine individualized exercise intensity, participants will complete a graded treadmill exercise test to volitional exhaustion approximately one week prior to the intervention. VO_2max will be determined using indirect calorimetry. Heart rate will be monitored to confirm maximal effort. The results will be used to calibrate treadmill speed for the main exercise session to target 70–80% of each participant's VO_2max. Participants will return to the lab after the VO_2max test for the main experimental trial. To reduce variability as much as possible, participants will be instructed to arrive in a fasted state (≥8 hours) and to refrain from exercise, alcohol, and caffeine for 24 hours prior to testing. After a brief warm-up, participants will run on a treadmill for 60 minutes at 70–80% of their VO_2max. This exercise intensity and duration are adapted from protocols shown to elicit robust VEGFA responses in skeletal muscle [17]. Muscle biopsies from the vastus lateralis will be collected at four time points: pre-exercise (PRE), immediately post-exercise (T0), 1-hour post-exercise (T1), and 3 hours post-exercise (T3). To minimize local inflammation, biopsies will be taken from alternating legs under local anesthesia. Biopsy timing is selected based on prior studies [17] demonstrating dynamic transcriptional and epigenetic changes in muscle tissue within this time window. Freshly obtained muscle biopsies will be immediately cleaned of visible fat and connective tissue. Samples designated for gene expression analysis will be snap-frozen in liquid nitrogen and stored at –80°C.

Chromatin Immunoprecipitation (ChIP)

ChIP experiments will be performed in accordance with previous experimenters [18]. Briefly, approximately 30 mg of vastus lateralis muscle tissue will be homogenized and cross-linked by incubating with 1% formaldehyde for 10 minutes at room temperature, followed by quenching with 125 mM glycine for 5 minutes. The resulting pellet will be washed with cold PBS and centrifuged at 12,000 g for 4 minutes. Samples will then be lysed in a buffer containing 50 mM Tris, 10 mM EDTA, and 1% SDS (pH 8.0). The chromatin will be sonicated to obtain DNA fragments between 200–700 bp in size. Sheared chromatin will be

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diluted in ChIP buffer (50 mM Tris, 167 mM NaCl, 1.1% Triton X-100, pH 8.0). Immunoprecipitation will be performed by incubating chromatin overnight at 4°C with an antibody targeting H3K27me3. The next day, protein A/G magnetic beads will be added and incubated at 4°C for 4 hours on rotation. Beads will be washed with low salt buffer (50 mM Tris, 1 mM EDTA, 150 mM NaCl, 0.1% SDS, 1% Triton X-100, pH 8.0) and high salt buffer (same formulation with 500 mM NaCl). Elution will be carried out with ChIP Elution buffer (10 mM Tris, 5 mM EDTA, 300 mM NaCl, 0.5% SDS, pH 8.0) at 65°C for 2-hours. RNAse A (20 µg) will be added and incubated at 37°C for 30 minutes, followed by Proteinase K treatment (0.6 mg/mL) at 65°C for 2 hours. ChIP DNA will be purified and eluted in nuclease-free water.

ChIP-qPCR

To assess H3K27me3 enrichment at the *VEGFA* promoter, primers will be designed to amplify a short region near the transcription start site (TSS). The reference sequence for *VEGFA* will first be obtained from the NCBI Gene database, focusing on ~1 kb upstream of the TSS. This region is selected to capture chromatin dynamics that may influence transcriptional activation. Using Primer3Web (https://primer3.ut.ee/), primers will be generated with the following constraints: amplicon size between 80–150 base pairs and an optimal melting temperature (Tm) near 60°C. The software will provide several primer options that meet these criteria. To ensure target specificity, candidate primer pairs will be validated using the UCSC In-Silico PCR tool. This step confirms that the primers selectively amplify the *VEGFA* promoter region and avoid off-target binding elsewhere in the genome. Once validated, the chosen primers will be used in SYBR Green-based RT-qPCR to measure VEGFA promoter enrichment for H3K27me3 on DNA isolated by ChIP. The percent input method will be used for ChIP-qPCR analysis [19].

mRNA Extraction and RT-qPCR

Total RNA will be extracted from ~15 mg of vastus lateralis muscle using a Fibrous Tissue RNA Extraction Kit. Samples will be homogenized using a tissue homogenizer and RNA will be extracted according to kit manufacturer's instructions. RNA purity and concentration will be assessed spectrophotometrically (260/280 nm). 500 ng of purified RNA will be reverse-transcribed into cDNA and RT-qPCR will be performed in triplicates using TaqMan Fast Advanced Master Mix and TaqMan probes specific to VEGFA and a housekeeping gene (e.g., HPRT). The PCR protocol includes 40 cycles of denaturation at 95°C for 3 seconds followed by annealing and extension at 60°C for 30 seconds. Gene expression data will be normalized to HPRT and quantified using the $2^{-\Delta\Delta Ct}$ method [20] with PRE (baseline) samples used as the reference condition.

Protein Extraction and Immunoblotting

Skeletal muscle protein extraction and immunoblotting will be performed as previously described [21]. Briefly, approximately, 15 mg of vastus lateralis muscle tissue will be homogenized in 15x volume of RIPA buffer (50 mM Tris-base, 100 mM NaCl, 5 mM EDTA, 1% sodium deoxycholate, 1% triton X-100, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM NaF, 1 mM Na3VO4). After incubating at 4°C for 20-minutes, the homogenate will be spun in a centrifuge for 15 min at 16,000g and 4°C. The supernatant will be extracted and used for immunoblotting analysis after protein concentration is determined by BCA. Immunoblotting will be performed on 20 µg of muscle tissue proteins. Samples will be diluted in a 1/5 vol. ratio of loading buffer (2.69 mM sucrose, 0.28M SDS, 2.84M β-mercaptoethanol, 14 µM bromophenol blue, 0.5 M Tris-base pH 6.8) separated by SDS-PAGE, and then blotted onto nitrocellulose membranes. After blocking with 5% fat-free milk in 0.1% tween tris-buffered saline buffer for 1-hour, membranes will be probed overnight at 4°C with antibodies specific to JMJD3 (KDM6B) and KDM4B. α/β-tubulin will be used as a loading control. Membranes will then be incubated with HRP (Horseradish Peroxidase) -conjugated antibodies specific to species used for primary antibodies. Proteins will be visualized using enhanced chemiluminescence (ECL) and images will be obtained using a chemiluminescence imaging station with multiple exposure times.

Statistical Analysis

Statistical analyses will be performed using 2-way ANOVA with Prism10 (GraphPad software) and Tukey multiple comparison post hoc for ANOVA analyses. $P < 0.05$ will be considered statistically significant.

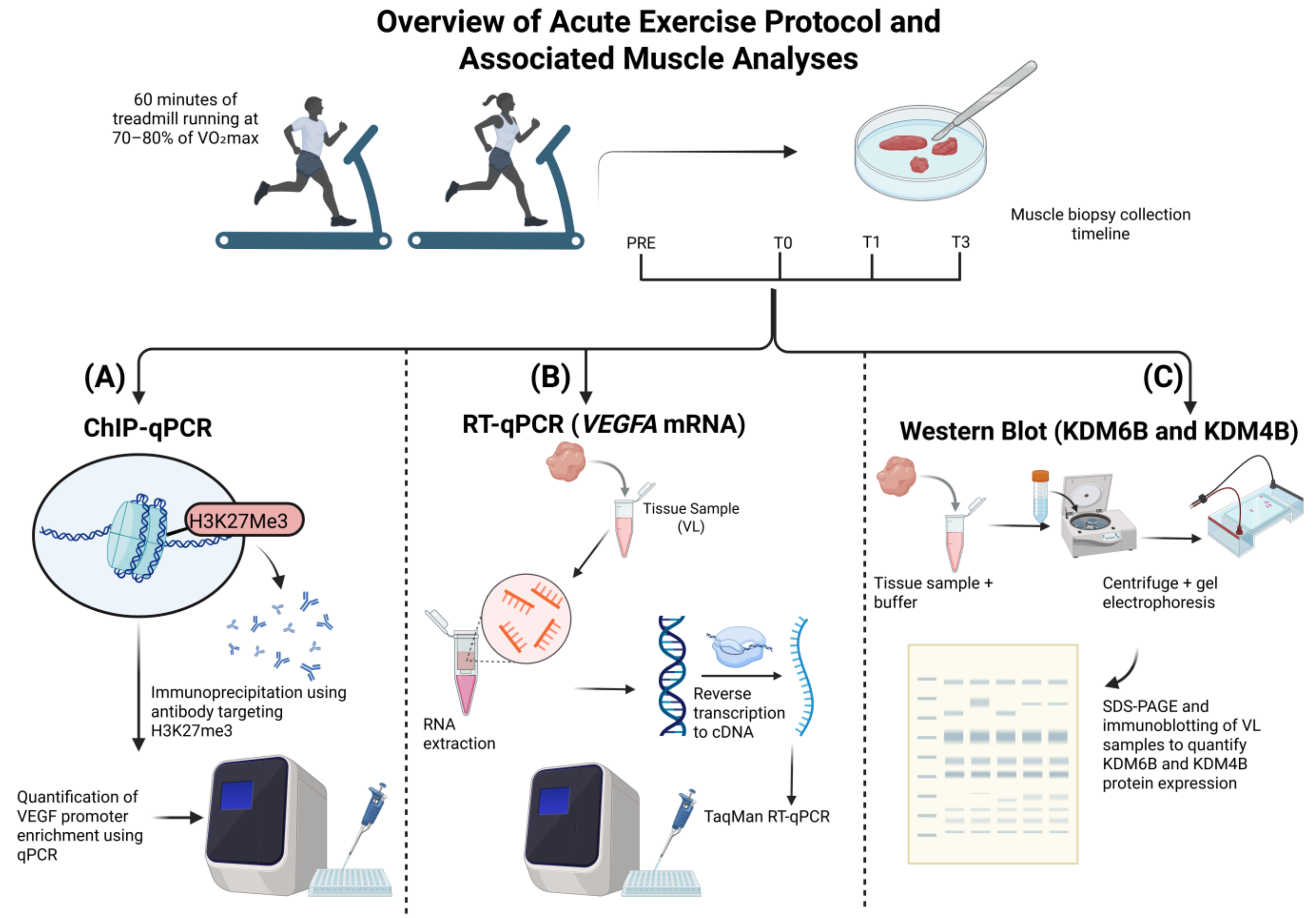

Figure 1. Overview of the Human Acute Exercise Protocol and Experimental Analyses Participants will complete a 60-minute treadmill run at 70–80% of their VO_2max. Skeletal muscle biopsies will be collected at four time points: pre-exercise (PRE), immediately post-exercise (T0), 1 hour post-exercise (T1), and 3 hours post-exercise (T3). Muscle samples will be processed for (A) ChIP-qPCR, to assess H3K27me3 enrichment at the *VEGFA* promoter; (B) RT-qPCR, to quantify *VEGFA* mRNA expression; and (C) Western blotting, to assess protein levels of histone demethylases JMJD3 (KDM6B) and KDM4B. This integrated design enables the investigation of epigenetic and transcriptional regulation of *VEGFA* in response to acute exercise. Created in https://BioRender.com

Results

We anticipate that our proposed experimental protocol will reveal dynamic changes in the chromatin structure at the VEGFA locus as well as VEGFA gene expression in response to acute exercise. Specifically, we expect ChIP-qPCR analysis to show the highest enrichment of the repressive H3K27me3 mark at the VEGFA promoter under resting conditions (PRE). Following exercise, we anticipate a progressive decrease in H3K27me3 enrichment at 1 hour (T1) and 3 hours (T3) post-exercise, reflecting chromatin remodeling toward a more permissive state for gene transcription (Figure 2A). In parallel, we expect VEGFA mRNA expression, assessed by RT-qPCR, to be elevated 1-hour (T1) after exercise and further increase by 3-hour (T3) post-exercise (Figure 2B). These results would be consistent with increased transcriptional activation driven by changes in chromatin accessibility via loss of the repressive effect of H3K27me3 on the VEGFA promoter.

We also anticipate that the protein expression of the histone demethylases, KDM6B (JMJD3) and KDM4B, would be upregulated in skeletal muscle post-exercise considering that their expression is stimulated in hypoxic endothelial cells. We expect JMJD3 and KDM4B protein expression to be rapidly increased immediately post-exercise (T0) and plateau through later time points (T1-T3) (Figure 3A, Figure 3B). These results would suggest that an immediate increase in demethylases responsible for removing the H3K27me3 silencing modification proceeds the loss of H3K27me3 enrichment on the VEGFA promoter allowing for subsequent transcription to occur.

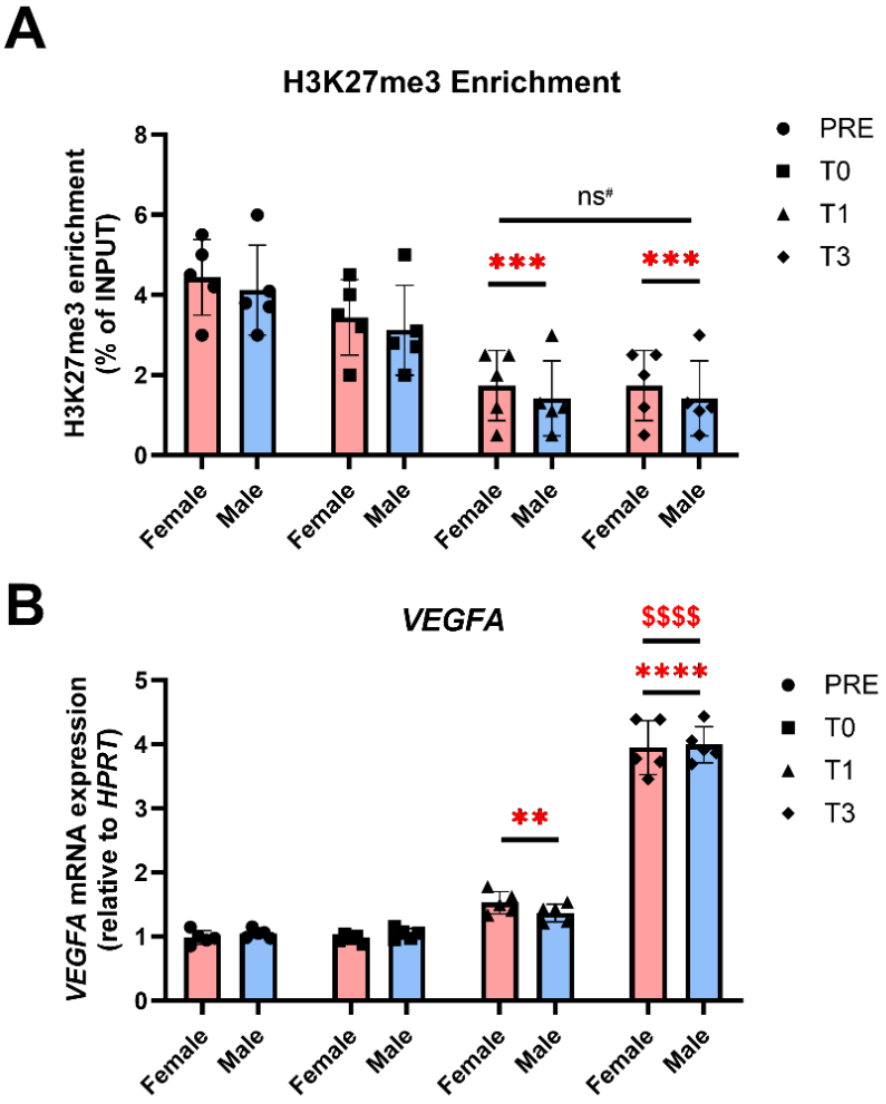

Figure 2. Acute endurance exercise decreases H3K27me3 enrichment and increases VEGFA mRNA expression in human skeletal muscle (A) Chromatin immunoprecipitation (ChIP) followed by qPCR revealed a significant reduction in H3K27me3 enrichment at the VEGFA promoter in both male and female participants at 1 hour (T1) and 3 hours (T3) post-exercise compared to pre-exercise (PRE). Two-way ANOVA with Tukey's post hoc revealed no significant difference was observed between sexes or between T1 and T3. Data represents means ± SD with n=5 subjects per group. Statistical significance relative to PRE: ***, P<0.001. ns# indicates no significant difference between T1 and T3. (B) VEGFA mRNA expression, measured by RT-qPCR and normalized to HPRT, significantly increased at 1 hour and was further elevated at 3 hours post-exercise, expressing a significant difference between T1 and T3. Two-way ANOVA with Tukey's post hoc revealed no significant difference was observed between sexes. Data represents means ± SD with n=5 subjects per group. Statistical significance relative to PRE: **, *P*<0.01; ****, *P*<0.0001. Statistical Significance relative to T1: $$$$, *P*<0.0001. Figure created with GraphPad Prism10.

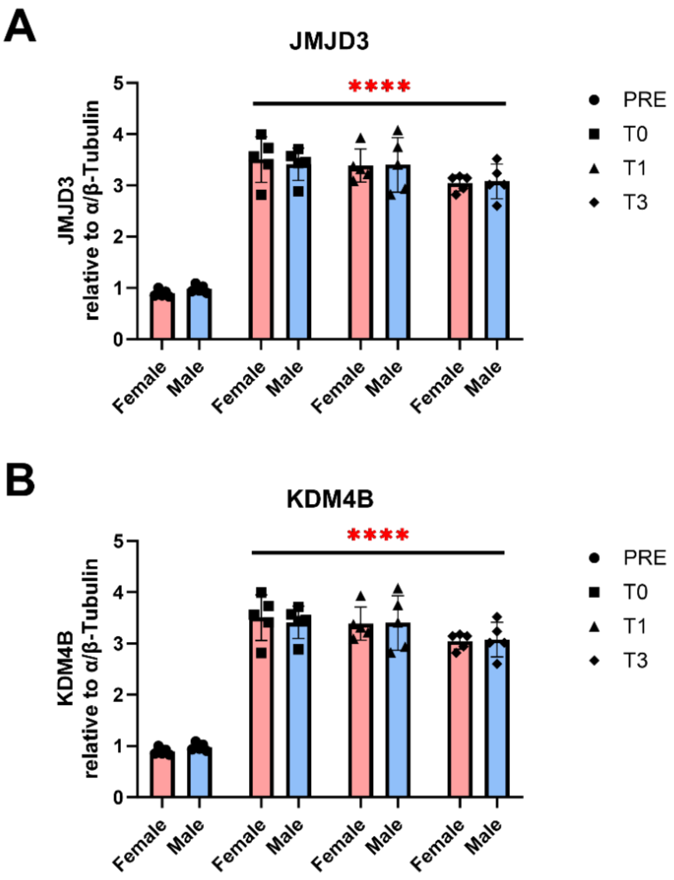

Figure 3. JMJD3 and KDM4B protein expression increase in human skeletal muscle following acute exercise (A) JMJD3 and (B) KDM4B protein levels were quantified via immunoblotting and normalized to α/β tubulin. Both demethylases showed a significant increase immediately post exercise (T0) compared to pre-exercise (PRE) and remained elevated at 1 hour (T1) and 3 hours (T3). Two-way ANOVA with Tukey's post hoc revealed no significant difference was observed between sexes. Data represents means ± SD with n=5 subjects per group. Statistical significance relative to PRE: ****, *P*<0.0001. Figure created with GraphPad Prism10.
Note: All results and figures presented are hypothetical and generated for the purpose of illustrating expected outcomes in this research proposal. No human participants were recruited, and no experimental procedures were conducted.

Discussion

Skeletal muscle is a dynamic tissue that undergoes robust molecular and tissular adaptations in response to endurance exercise, including angiogenesis. A key regulator of this process is VEGFA, whose transcription is strongly induced by hypoxia via stabilization of HIF-1α [9, 15]. In skeletal muscle, acute endurance exercise imposes a metabolic and oxygen demand that transiently reduces cellular oxygen tension, resulting in a localized hypoxic environment. This hypoxic environment stabilizes HIF-1α, a key transcription factor that promotes VEGFA to support angiogenic adaptation. Numerous studies have documented a rapid increase in VEGFA mRNA expression following acute exercise in human and rodent skeletal muscle, typically peaking within hours post-exercise [9, 10, 15]. Yet beyond this canonical pathway emerging evidence in endothelial cells suggests that hypoxia induction of VEGFA is also modulated epigenetically through changes in chromatin structure. In particular, the removal of the repressive histone modification, H3K27me3, has been shown to facilitate VEGFA transcriptional activation [16]. This demethylation is mediated by oxygen-sensitive histone demethylases, KDM6B (JMJD3) and KDM4B. It remains unclear however, whether similar mechanisms operate in skeletal muscle myocytes during exercise-induced hypoxia. This study aimed to investigate whether acute aerobic exercise in humans triggers epigenetic remodeling at the VEGFA promoter through the loss of H3K27me3 and whether this process is associated with increased expression of key histone demethylases.

To explore this hypothesis, we propose a human acute exercise protocol involving recreationally active males and

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females. Participants will perform 60 minutes of treadmill running at 70–80% of their VO_2max, a workload previously shown to elicit a strong VEGFA transcriptional responses in skeletal muscle [17]. Muscle biopsies from the vastus lateralis will be collected at four time points: pre-exercise (PRE), immediately post-exercise (T0), 1 hour post (T1), and 3 hours post (T3). To evaluate chromatin dynamics at the VEGFA promoter, chromatin immunoprecipitation followed by quantitative PCR (ChIP-qPCR) will be performed using an antibody specific to H3K27me3. In parallel, mRNA levels of VEGFA and the protein expression of histone demethylases KDM6B (JMJD3) and KDM4B will be measured using RT-qPCR and immunoblotting, respectively. This integrated design will allow us to assess whether acute exercise leads to epigenetic remodeling that coincides with transcriptional activation of VEGFA and upstream chromatin modifiers.

Our proposed results would support the idea that histone modifications are critical for muscle phenotype. We hypothesized that acute exercise would reduce H3K27me3 enrichment at the VEGFA promoter enabling the transcription of this key angiogenic gene needed for vascular remodelling of the skeletal muscle. This epigenetic regulatory axis would purportedly contribute to the scientific knowledge of the importance of histone modifications in driving functional adaptations of the skeletal muscle, in our context angiogenesis. Kawano et al. (2015) show that histone modifications are important for muscle contractile phenotype by revealing distinct epigenetic landscapes between fast- and slow-twitch muscles [22]. Specifically, they found that transcriptionally active histone marks (H3K4me3, H3Ac) are enriched in fast-twitch gene loci in fast muscle (plantaris) but not similarly enriched in slow-twitch genes in slow muscle (soleus), even when those genes were highly expressed. These results, in addition to our proposed findings, would suggests that histone modifications play a key role in defining muscle vascular and contractile phenotype.

Exercise is an extremely powerful stimulus in driving positive adaptation of the muscle tissue. Here, we propose that acute exercise transiently alters H3K27me3 enrichment on the genome by demonstrating a time-dependent decrease in H3K27me3 levels at the VEGFA promoter following exercise, as measured by ChIP-qPCR. Shimizu and Kawano demonstrated acute exercise to dynamically alter H3K27me3 enrichment across the genome in skeletal muscle, with increased H3K27me3 observed at transcriptionally active loci following a single bout of exercise [23]. Using pharmacological inhibitors, they showed that modulating H3K27me3 levels directly influences gene expression and muscle adaptation. Whilst our hypothesized results would not directly align with Shimizu and Kawano, they do share an underlying common theme of exercise dramatically altering skeletal muscle chromatin dynamics via H3K27me3. Supporting our hypothesized results is fact that exercise induces a hypoxic environment within the skeletal muscle. If we consider the results from Liu et al., that hypoxia reduces H3K27me3 enrichment on the VEGFA promoter in endothelial cells it is not impossible for a similar mechanism to be present in myocytes. Furthermore, in our study we propose to use biopsies from vastus lateralis muscle. The skeletal muscle is a heterogenous tissue composed of multinuclear myofibers and mononuclear cells, including endothelial cells [24]. It is not outside of the realm of possibility that our proposed results could be an artefact of hypoxic stress on resident skeletal muscle endothelial cells.

Finally, histone modifications are not static but rather change dynamically in response to external stimuli and perturbations. In this context, we proposed to investigate how exercise may affect the expression of histone modifying enzymes relevant to our scientific question. We hypothesized acute exercise to reduce H3K27me3 abundance at the VEGFA promoter via stimulating KDM6B (JMJD3) expression. This would support the idea that exercise facilitates integrative changes between histone marks and the enzymes that regulate them to facilitate transcriptional adaptation in skeletal muscle. McGee et al. (2009) demonstrated that histone modifying enzymes are sensitive to acute exercise by showing that a single bout of endurance cycling triggered the phosphorylation and nuclear export of two class IIa histone deacetylase, thereby relieving their transcriptional repression and promoting gene expression in skeletal muscle [21]. Furthermore, aligned with our proposal, Li et al. (2024) demonstrated endurance exercise training to remodel histone methylation in skeletal muscle due to increased JMJD3 expression and reductions in H3K27me3 [18]. These epigenetic changes were associated with increased mitochondrial biogenesis and a shift from fast- to slow-twitch muscle fibers.

Conclusion

This study demonstrates that epigenetic regulation of VEGF via H3K27me3 demethylation occurs in human skeletal muscle following acute endurance exercise, indicating that this chromatin-based mechanism is not exclusive to endothelial cells. The coordinated decrease in H3K27me3 enrichment at the VEGF promoter, alongside increased expression of the histone demethylases KDM6B and KDM4B and elevated VEGF mRNA levels, suggests that myocytes employ an oxygen-sensitive epigenetic program to regulate angiogenic gene expression in response to exercise-induced hypoxia. These findings underscore the broader role of histone modifications in skeletal muscle plasticity and vascular adaptation. Future studies are warranted to explore whether there are sustained epigenetic changes at angiogenic loci and whether manipulating histone demethylases can modulate exercise outcomes in clinical or athletic settings.

List of Abbreviations

BCA: bicinchoninic acid
ChIP: chromatin immunoprecipitation
ChIP-qPCR: chromatin immunoprecipitation followed by quantitative polymerase chain reaction
ECL: enhanced chemiluminescence
EDTA: ethylenediaminetetraacetic acid
HIF-1α: hypoxia inducible factor 1 alpha
HIF-1β: hypoxia inducible factor 1 beta
HRE: hypoxia response element
HRP: horseradish peroxidase
JMJD3: jumonji domain-containing 3 (synonym for kdm6b)
KDM6B / KDM4B: lysine demethylase 6b / 4b
Na_3VO_4: sodium orthovanadate
NaF: sodium fluoride
PARQ: physical activity readiness questionnaire
PBS: phosphate buffered saline
PMSF: phenylmethylsulfonyl fluoride
RIPA: radioimmunoprecipitation assay buffer
RT-qPCR: reverse transcription quantitative polymerase chain reaction
SDS: sodium dodecyl sulfate
SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis
TSS: transcription start site
VEGF / VEGFA: vascular endothelial growth factor A
VO_2max: maximal oxygen uptake (volume of oxygen max)

Conflicts of Interest

The author(s) declare that they have no conflict of interests.

Ethics Approval and/or Participant Consent

This manuscript represents a research proposal. All results presented are hypothetical and no human testing, biopsies, or data collection were performed. As such, ethics approval was not required.

Authors' Contributions

ZS: Made substantial contributions to the conception and design of the study, drafted and revised the manuscript critically for important intellectual content, approved the final version to be published, and agrees to be accountable for all aspects of the work.

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