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Original Article

Identification of differentially expressed genes in resting human skeletal muscle of sedentary versus strength and endurance-trained individuals using bioinformatics analysis and in vitro validation

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Abstract

Understanding the molecular mechanisms underlying skeletal muscle adaptation to different training regimens is essential for advancing muscle health and performance interventions. The aim of this study was to investigate molecular and genetic adaptations in the resting skeletal muscle of sedentary individuals compared to strength- and endurance-trained athletes using bioinformatics and in vitro validation. Differentially expressed genes (DEG) analysis of the GSE9405 dataset was conducted. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed, followed by protein-protein interaction (PPI) network analysis and receiver operating characteristic (ROC) analysis. To validate the bioinformatics findings, the expression of two identified genes was assessed using real-time polymerase chain reaction (PCR) in professional athletes and age-matched non-athletes. Analysis of RNA expression profiles from the GSE9405 dataset identified 426 DEGs, with 165 upregulated and 261 downregulated in trained individuals. Enrichment analysis highlighted pathways related to metabolic efficiency, mitochondrial function, and muscle remodeling, all crucial for athletic performance. *PRKACA* and *CALM3* were identified as key upregulated genes in trained individuals with central roles in these pathways. The area under the curve (AUC) values for *CALM3* and *PRKACA* were 0.8558 and 0.8846, respectively, for differentiating the two groups. Validation in human samples confirmed that *CALM3* expression was significantly higher in athletes (p=0.001), suggesting its critical role in muscle adaptation. However, *PRKACA* expression differences between the groups were not statistically significant (p=0.321). These findings provide insights into gene-level responses to long-term training, offering a basis for targeted interventions to enhance muscle health and athletic performance.

Keywords: Skeletal muscle, gene expression regulation, physical endurance, *CALM3*, *PRKACA*

Introduction

In the field of sports and athletic performance, a comprehensive understanding of skeletal muscle physiology is crucial [1]. Skeletal muscle not only facilitates physical movement but also

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plays a vital role in metabolic health and recovery [2]. For athletes, optimizing skeletal muscle function is key to achieving peak performance, preventing injuries, and ensuring efficient post-exercise recovery [3]. While much attention has been given to muscle adaptations during exercise, the resting state of skeletal muscle is equally important. This state reflects the long-term effects of various training regimens and provides insights into the muscle's preparedness for future physical demands. The condition of resting skeletal muscle can reveal the enduring impacts of training, particularly in athletes who undergo intensive strength and endurance training [4].

However, investigating the molecular adaptations in resting skeletal muscle presents significant challenges. Traditional approaches, such as muscle biopsy followed by in vitro analysis, are invasive, resource-intensive, and difficult to apply to large cohorts, especially when comparing sedentary individuals with highly trained athletes. These limitations have contributed to a gap in the literature, where the understanding of how long-term training influences gene expression in resting muscles remains incomplete. Furthermore, variability in sample collection and the complexity of post-exercise muscle conditions further complicate direct studies [3].

To address these challenges, bioinformatics offers a powerful and non-invasive alternative. By analyzing existing gene expression datasets, researchers can explore the molecular landscape of skeletal muscle without the need for direct sample collection [5]. Bioinformatics enables the identification of differentially expressed genes (DEGs) across various conditions, providing a comprehensive overview of the molecular changes associated with different training regimens [6]. This approach not only helps bridge the current knowledge gap regarding resting muscle adaptations but also serves as a foundational study for future in vitro experiments, where specific genes identified in this analysis can be further validated and explored in samples from both athletes and non-athletes.

In this study, bioinformatics tools were utilized to analyze the GSE9405 dataset from the Gene Expression Omnibus (GEO) database. The aim of this study was to identify and characterize the key genes that exhibit differential expression in resting skeletal muscle between sedentary and highly trained individuals. By addressing this gap, the research aims to provide critical insights into the molecular adaptations of skeletal muscle in response to different training regimens, ultimately laying the groundwork for subsequent in vitro investigations. These insights could significantly contribute to the development of targeted interventions for enhancing athletic performance and muscle health.

Methods

Data acquisition and differential expression analysis

The RNA expression profile of GSE9405 (https://ncbi.nlm.nih.gov/bioproject/103133) was obtained from the GEO database (https://www.ncbi.nlm.nih.gov/geo/) using the R package GEOquery (v2.66.0) [7]. The R package limma (v3.54.2) was used for normalization and DEGs, applying criteria of fold change (FC) >1 and adjusted $p<0.05$.

Functional enrichment analysis

Gene Ontology (GO) (https://www.geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.genome.jp/kegg/) pathway analyses were performed on the identified DEGs to understand the biological processes, cellular components, and molecular functions associated with skeletal muscle adaptation. These analyses were conducted using the "clusterProfiler" of the R package (v4.6.2) with a significance level of $p<0.05$.

Protein-protein interaction (PPI) network construction and hub gene identification

The identified DEGs were used to construct a PPI network utilizing the STRING database (https://stringdb.org/) with a confidence score >0.4. The PPI network was visualized using Cytoscape (v3.10.2) (https://www.softpedia.com/get/Science-CAD/Cytoscape.shtml), where unconnected nodes were removed, and hub genes were identified based on node degree (Degree>20). Finally, the R package pROC (v1.18.5) was employed for receiver operating characteristic (ROC) analysis, including the generation of ROC curves.

In vitro validation

To validate bioinformatics findings, venous blood samples were collected from two groups: professional athletes and age-matched non-athletes. The criteria for professional athletes were as follows: male, aged 18 to 40 years, regularly training in strength and endurance. Professional athletes included eight physical education and health students at Universitas Negeri Malang and one basketball player. The criteria for non-athletes were male, aged 18 to 40 years, with no training in strength and endurance. Eight students of the literature department at Malang State University were included in the non-athlete group.

RNA was extracted from blood samples using a TRIzol-based protocol (TRIzol RNA Isolation Reagents, Thermo Fisher Scientific, Waltham, USA), followed by reverse transcription to synthesize complementary DNA (cDNA). Polymerase chain reaction (PCR) was performed to assess the expression levels of the *CALM3* and *PRKACA* genes. These genes were identified through our bioinformatics analysis. *CALM3* and *PRKACA* play critical roles in muscle growth, differentiation, and adaptation. *CALM3* is primarily involved in heart muscle contraction, while *PRKACA* expression is upregulated in skeletal muscle tissue and is essential for cardiac muscle contraction. Gene-specific primers were synthesized by PT Genetika Science Indonesia (Tangerang, Indonesia) (**Table 1**).

Table 1. Primer sequences used for real-time polymerase chain reaction (PCR)

Gene	Primer sequence
CALM3	Forward: ACT TTC GCC TCT GGG ACA AG
	Reverse: TGC TGG GGT TGG TGT GTA TG
PRKACA	Forward: TAG GAA GCC TCC GCT CTC TT
	Reverse: TTC AAG CCA GGA CCT GAT CG
ACTB	Forward: GAG CTG TCA CAT CCA GGG TC
	Reverse: AGG AGG GAT CTC CAC ACA CC

The PCR was conducted using the DNA Engine Opticon (Bio-Rad Laboratories, Hercules, California). Each reaction had a volume of 20 µL with 4 µL of template, 1 µL of each forward and reverse primer (0.5 µM), 4 µL PCR master mix (Promega Corporation, Madison, USA) (2 mg/mL) and 10 µL of PCR grade water. The amplification conditions included an initial denaturation at 94°C for 2 minutes, followed by 40 cycles of denaturation at 94°C for 30 seconds and annealing at 60°C for 30 seconds. Gene expression was normalized against the housekeeping gene beta-actin (*ACTB*) to ensure accurate and reliable results. The relative expression levels of *CALM3* and *PRKACA* were calculated based on densitometric analysis [8]. Statistical analysis of gene expression data was performed using SPSS v21.0 (IBM, New York, USA), with a significant threshold of $p<0.05$.

Results

Differentially expressed genes (DEG) analysis

The DEG analysis of resting skeletal muscle between sedentary individuals and highly trained athletes reveals significant molecular adaptations to athletic training. Utilizing the RNA expression profile from the GEO dataset GSE9405, a total of 426 genes were identified as differentially expressed (**Figure 1**). Among these, 165 genes were significantly upregulated, and 261 genes were significantly downregulated in the muscles of highly trained individuals compared to sedentary individuals (**Figure 1**). The upregulated genes (red dots), likely involved in enhanced metabolic processes, increased mitochondrial function, and other adaptations that support the high demands of athletic training. This upregulation indicated that the muscles of highly trained individuals were primed for efficient energy utilization and recovery, even at rest. The downregulated genes may reflect the suppression of pathways that are less essential in a well-conditioned muscle, such as those involved in inflammation, oxidative stress, or other mechanisms that are more active in sedentary individuals. The greater number of downregulated genes suggested extensive muscle remodeling in athletes, leading to a more specialized and efficient state at rest. Most of the genes (15,990; gray dots) did not exhibit significant differential expression, likely representing baseline molecular processes common to both groups (**Figure 1**).

This pattern indicated the profound yet selective impact of athletic training on the muscle transcriptome, where significant changes occur alongside a stable baseline of gene expression.

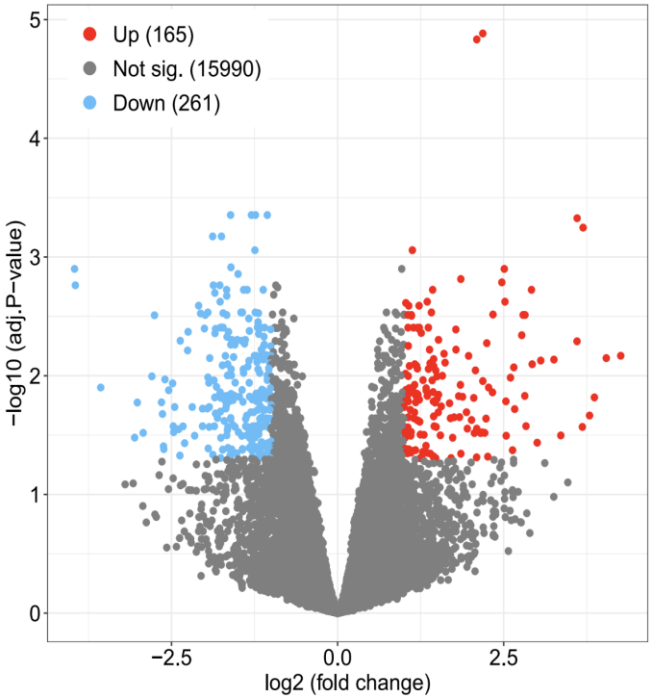

Figure 1. Volcano plot of differentially expressed genes (DEGs) in resting skeletal muscle between sedentary individuals and highly trained athletes. Blue dots represent 261 downregulated DEGs, and red dots represent 165 upregulated DEGs. The gray dots represent 15,990 genes that do not exhibit significant differential expression.

Gene Ontology (GO) enrichment analysis

The GO enrichment analysis of resting skeletal muscle between sedentary individuals and highly trained athletes revealed substantial molecular differences as presented in **Figure 2**. Several biological processes were notably enriched in athletes, emphasizing muscle systems' heightened activity and efficiency. Muscle contraction, cardiovascular function, and DNA repair pathways were significantly upregulated in athletes, indicating enhanced muscular performance, improved cardiac efficiency, and heightened genomic stability (**Figure 2**). This was further supported by the significant enrichment of processes related to telomere organization, which suggested a role in preserving chromosome integrity during physical stress (**Figure 2**).

Muscle system processes, which included muscle adaptation, contraction, and hypertrophy, showed particularly significant differences between the training and control groups. This highlighted the enhanced muscle activity and growth in athletes compared to sedentary individuals. Additionally, the enrichment of telomere organization processes in athletes was particularly interesting, as telomere organization is closely related to telomerase activity, which plays a crucial role in maintaining chromosomal integrity under physiological stress. These findings suggest that while chronological age does not affect skeletal muscle cell aging, physical inactivity, potentially mediated by free radicals, has a profound impact on this process.

Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis

The KEGG enrichment analysis revealed significant molecular adaptations in the resting skeletal muscle of highly trained athletes compared to sedentary individuals as presented in **Figure 3**. Notably, the enrichment of pathways associated with dilated and hypertrophic cardiomyopathy suggested that athletic training induced molecular changes that may reflect adaptive cardiac remodeling. These adaptations could be protective, enhancing cardiac function and resilience in response to the increased physical demands experienced by athletes.

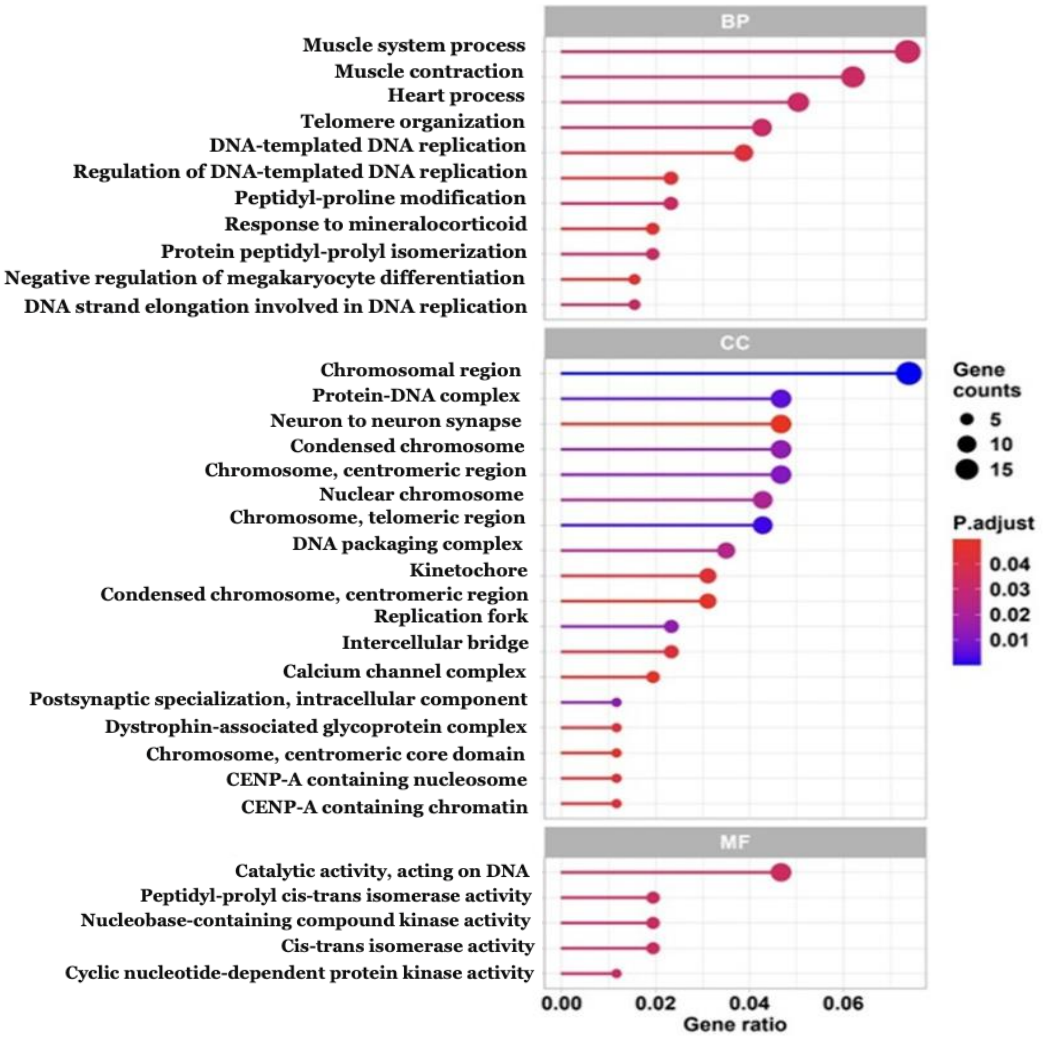

Figure 2. Gene Ontology (GO) enrichment analysis of resting skeletal muscle between sedentary individuals and highly trained athletes revealed significant molecular adaptations to athletic training, false discovery rate (FDR) <0.05.

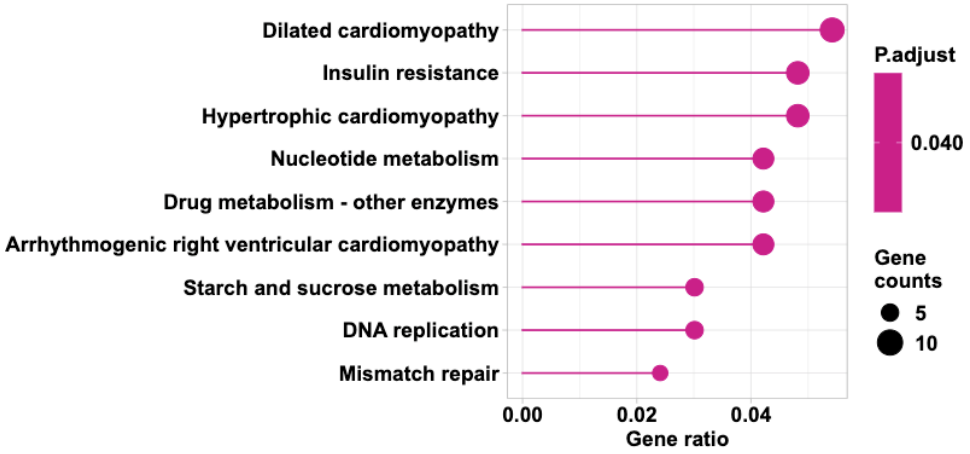

Figure 3. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of resting skeletal muscle between sedentary individuals and highly trained athletes revealed significant molecular adaptations to athletic training.

Protein-protein interaction (PPI) network

This study conducted a detailed analysis of PPI network to elucidate the differences in resting skeletal muscle between sedentary individuals and highly trained athletes. The PPI network,

derived from high-throughput data, highlighted several key proteins acting as central hubs, which play crucial roles in muscle physiology and adaptation. Proteins such as proto-oncogene tyrosine-protein kinase SRC (SRC), myelocytomatosis oncogene (MYC), enhancer of zeste homolog 2 (EZH2), and cyclin-dependent kinase 2 (CDK2) were identified as highly connected nodes within the network, indicating their significant involvement in regulating muscle function (**Figure 4**).

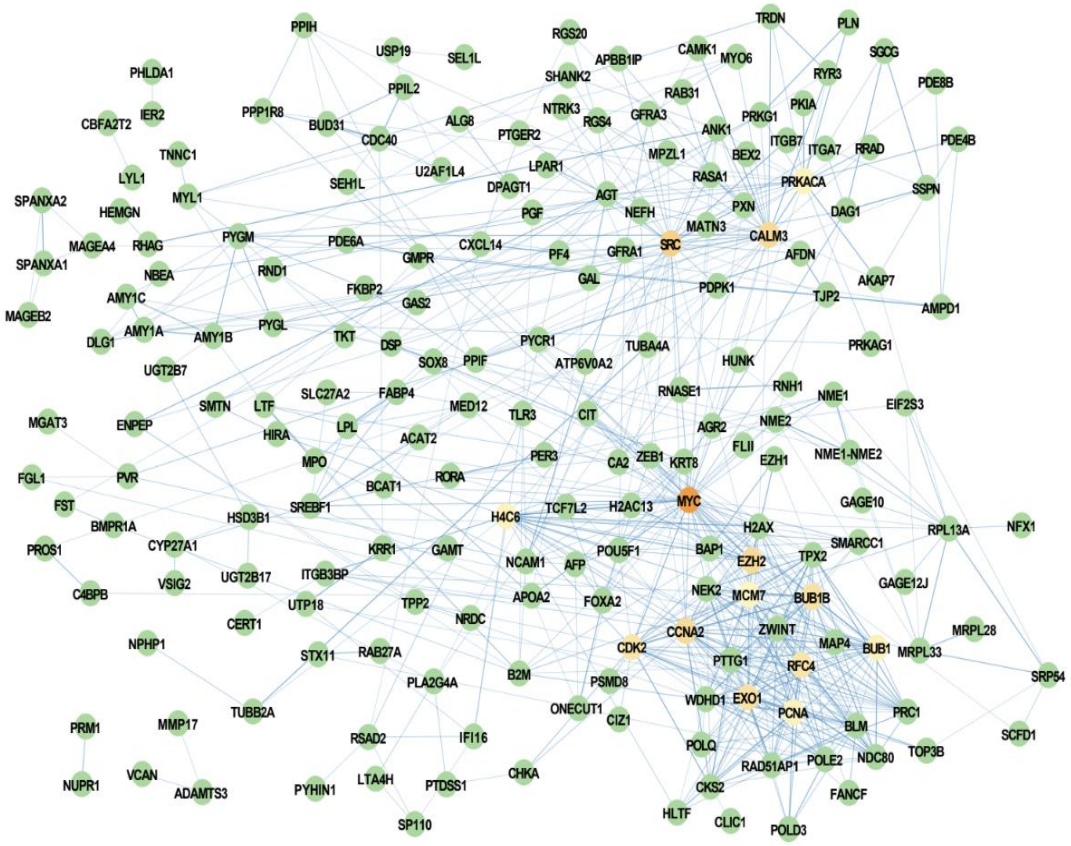

Figure 4. Protein-protein interaction (PPI) network of differentially expressed genes (DEGs). Nodes are color-coded based on degree: green indicates nodes with a degree less than 20, while yellow to orange indicates nodes with a degree greater than 20 (the more orange, the higher the degree). Edge width corresponds to interaction scores, with wider edges representing higher interaction scores.

Hub genes

Differential gene expression in resting skeletal muscle between sedentary (control) and trained (highly active) groups emphasized changes in key regulatory genes such as *MYC, CDK2, EZH2, SRC*, and *CALM3* (**Figure 5**). These genes are crucial for muscle growth, differentiation, and adaptation. *CALM3* and *PRKACA* genes had increased expression in the trained group, suggesting an adaptive upregulation in response to physical training. In contrast, genes like *MYC* and *CDK2* were downregulated, indicating a modified regulatory role in response to sustained exercise. Hierarchical clustering applied to genes and samples, as presented by the dendrograms, highlighted distinct expression profiles between the groups and demonstrated variability within them, especially for *EZH2* and *SRC* (**Figure 5**).

Next, the analysis was extended by mapping the network of genes central to muscle contraction, specifically highlighting *CALM3* and *PRKACA* (**Figure 6**). This network visualization revealed the direct involvement of these upregulated genes in the muscle contraction process, suggesting their functional significance in trained muscles. *CALM3* had significant upregulation and suggested an increased role in muscle contraction mechanisms post-training (**Figure 6**). *PRKACA* also showed enhanced expression, pointing to its critical role in muscle contraction regulation via interactions with other contraction-related proteins (**Figure 6**). These showed that the differential expression of these genes was not merely a reactive adjustment but actively enhanced muscle contraction capabilities in trained individuals, suggesting targeted molecular adaptations contributing to improved muscle function.

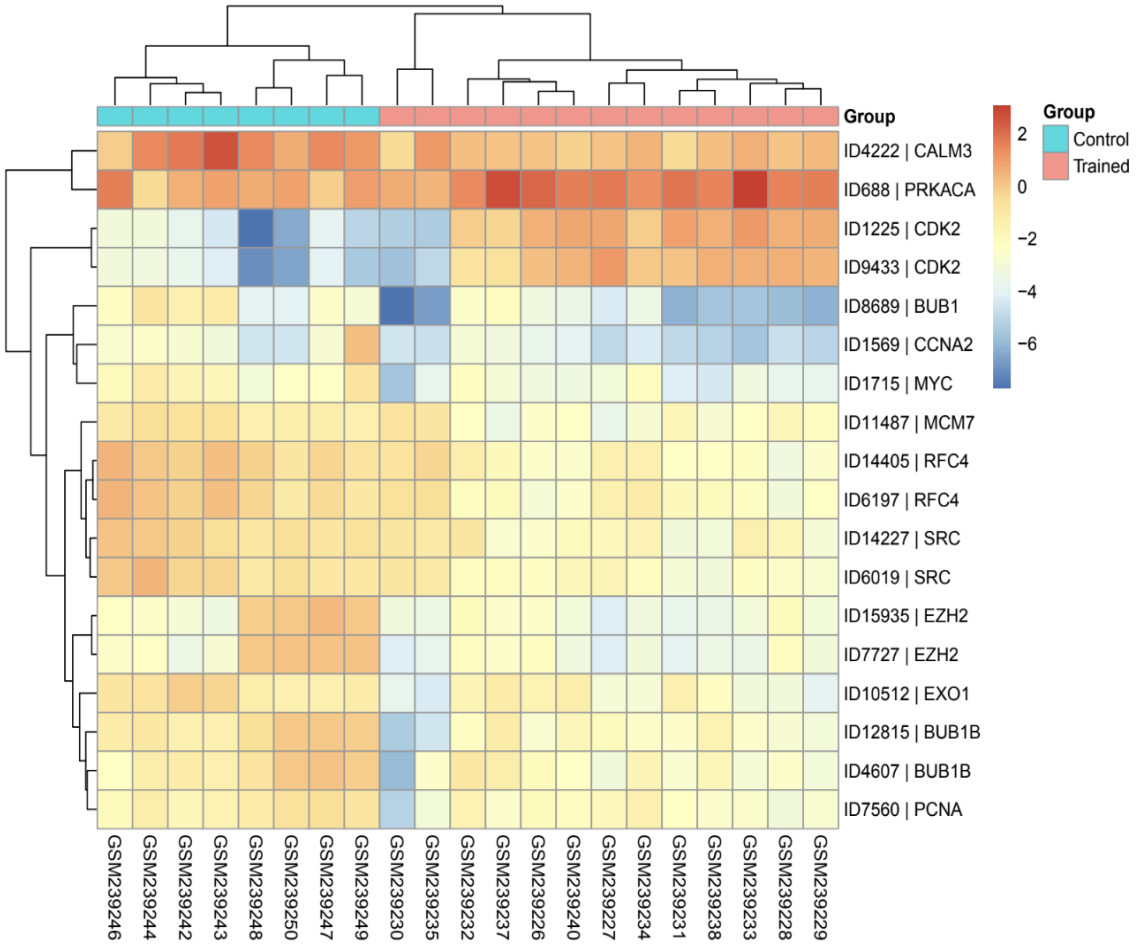

Figure 5. Heatmap of hub genes. The heatmap presents the hub genes' normalized log2 gene expression levels, with color intensity indicating gene abundance.

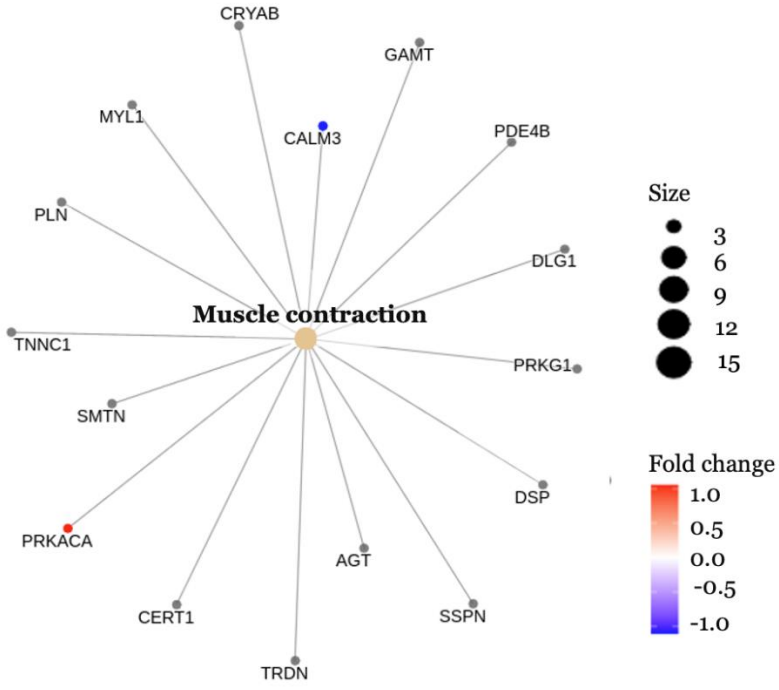

Figure 6. Hub genes and other differentially expressed genes (DEGs) are involved in the Gene Ontology term "Muscle contraction." The figure highlights the genes associated with the muscle contraction, distinguishing between hub genes and other DEGs. *CALM3* is presented in blue and *PRKACA* in red.

Receiver operating characteristic (ROC) analysis

In previous analyses, both *PRKACA* and *CALM3* were identified as differentially expressed genes, with upregulation observed in highly trained individuals. Our data indicated that *CALM3* and *PRKACA* had an area under the curve (AUC) values of 0.8558 and 0.8846, respectively, indicating that both genes could differentiate between the groups (**Figure 7**). The high AUC values in this ROC analysis further emphasized the diagnostic potential of these genes. The AUC values indicated that *PRKACA* and *CALM3* have strong discriminatory power in distinguishing between the sedentary and trained groups, reinforcing their importance in muscle function adaptation. The elevated expression of these genes in trained individuals, highlighted by their position in the network visualization, aligned with their high AUC values, confirming their critical roles in the physiological response to exercise.

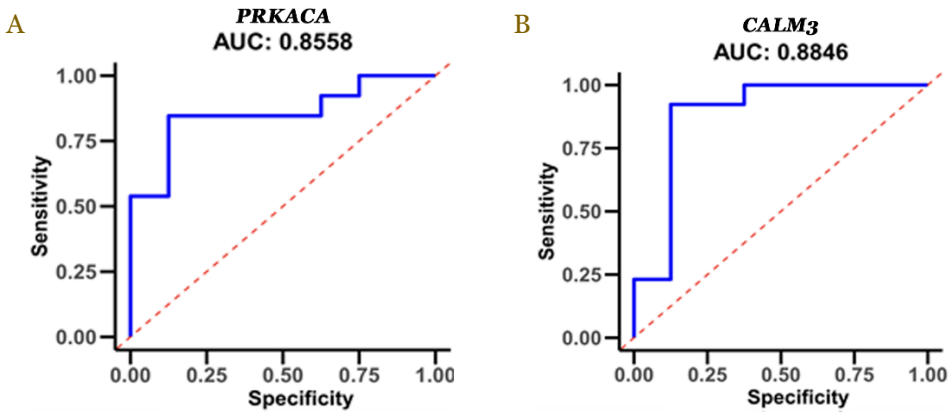

Figure 7. Receiver operating characteristic (ROC) curves of *PRKACA* (A) and *CALM3* (B) hub genes. The ROC curves depicted the performance of *PRKACA* and *CALM3* in distinguishing between sedentary and trained groups, with area under the curve (AUC) indicating predictive accuracy.

CALM3 and *PRKACA* gene expression

In this study, the expression of *CALM3* and *PRKACA* genes in athletes versus non-athletes was conducted using PCR with β-actin serving as the endogenous control. The results were expressed in the density of the PCR product obtained [8]. The expression results of each gene obtained were normalized against β-actin expression. Our analysis revealed the relative expression of the *CALM3* gene in the athlete group was significantly higher compared to the non-athlete group (p=0.01) (**Figure 8**). This indicated that the presence of moderate and high-intensity exercise affected the expression of the *CALM3* gene. In contrast, the *PRKACA* gene expression had no statistical significance between athlete and non-athlete groups (p=0.608).

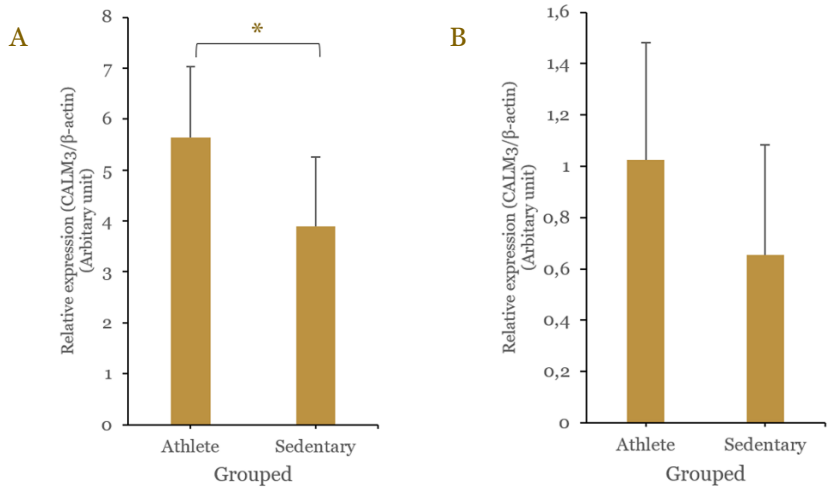

Figure 8. Comparison of densitometric analysis of relative expression of *CALM3* (A) and *PRKACA* gene (B) between athlete and non-athlete groups. The asterisk (*) symbol indicates statistical significance at p=0.05.

Discussion

Endurance training induces complex molecular adaptations, with more genes downregulated than upregulated. Notably, genes involved in lipid metabolism, like *SCD1* and *ATGL*, show higher expression in trained athletes compared to sedentary individuals. This enhances lipid metabolism, supporting prolonged physical activity by improving energy production and membrane integrity [9,10]. Exercise-induced changes in skeletal muscle reveal significant metabolic remodeling. Endurance training boosts oxidative capacity by increasing proteins linked to mitochondrial function and energy production. It also enhances fatty acid metabolism, promoting more efficient energy use. These changes improve both athletic performance and overall metabolic health [11,12].

Skeletal muscle acts as an endocrine organ, releasing myokines that regulate glucose and lipid metabolism, improving overall metabolic health. Exercise boosts insulin sensitivity by altering lipid profiles in muscle, underscoring its role in reducing metabolic disorder risks. Additionally, trained athletes exhibit muscle fiber adaptations, including enhanced oxidative capacity, efficient energy use, and increased lipid storage through *SCD1* upregulation, highlighting the intricate balance of muscle repair, growth, and metabolism [13-16]. Exercise training also modulates inflammatory markers in muscle tissue, as demonstrated by studies on the PGE2/COX pathway [17]. This modulation is particularly beneficial for sedentary individuals, as chronic inflammation is often linked to metabolic dysfunction. Furthermore, moderate and high-intensity aerobic training improves microvascular reactivity and reduces oxidative stress, which are critical factors in maintaining cardiovascular health [18]. These improvements are complemented by increases in skeletal muscle mitochondrial density, which enhances lipid oxidation and overall metabolic health [19].

Athletes' muscles undergo extensive cellular remodeling to support growth, repair, and performance. Training enhances transcriptional activity, chromosomal stability, and protein synthesis, strengthening muscle tissue and improving integrity [20]. Neuromuscular communication is optimized through enriched calcium channels and synaptic functions, boosting muscle contraction and performance. Additionally, increased DNA repair, protein folding, and stability enable better muscle maintenance and efficient energy use, highlighting the resilience and adaptability of trained muscles [21]. Athletic training uniquely impacts insulin sensitivity, often displaying the "athlete's paradox." Unlike typical insulin resistance associated with poor health, athletes may have higher intramyocellular lipid (IMCL) levels while maintaining or improving insulin sensitivity, reflecting an adaptive mechanism that boosts metabolic efficiency [14]. Research indicates that endurance-trained athletes possess higher levels of diacylglycerol (DAG) and IMCL than sedentary individuals, yet they demonstrate normal insulin sensitivity [9]. This is attributed to these lipids' distinct composition and localization within muscle cells. For instance, specific species of DAG have been shown to correlate positively with insulin sensitivity in athletes, suggesting that not all lipid accumulation is detrimental [10,22]. Furthermore, the presence of perilipin proteins involved in lipid droplet metabolism has been associated with improved insulin sensitivity in trained individuals [23].

Regular intense exercise enhances glucose utilization and insulin sensitivity through mechanisms like upregulated lipolytic proteins that lower muscle triglycerides, benefiting insulin signaling [24,25]. Athletes' metabolic flexibility ensures efficient glucose use despite elevated lipid levels, though stopping training quickly can impair metabolic health, emphasizing the need for consistency. Athletic training also enriches nucleotide metabolism, supporting the energy production, DNA repair, and cellular growth required for muscle repair and hypertrophy. Muscle growth is driven by the mTOR signaling pathway, activated by resistance exercise and nutrients like BCAAs, underscoring the intricate link between metabolism and training adaptations [26]. During muscle hypertrophy, genes linked to protein synthesis and metabolism are highly active. Resistance training triggers mTORC1 activation, sustained by BCAAs, and promotes growth factors like IGF-1 and testosterone, enhancing muscle protein synthesis [26–28]. Metabolic byproducts like lactate also create an anabolic environment that supports growth. Athletes show improved drug metabolism, aiding recovery and reducing oxidative stress. While intense exercise generates high ROS levels, upregulated antioxidant pathways adapt to protect DNA and maintain muscle integrity. Testosterone plays a dual role, boosting muscle growth and acting as an

antioxidant, essential for recovery and performance. Exercise-induced metabolic adaptations strengthen antioxidant defenses, reduce oxidative damage and support high-intensity training. However, the misuse of anabolic steroids raises ethical concerns in sports medicine due to their impact on performance and metabolic balance [29].

The molecular regulation of muscle hypertrophy is further driven by key signaling molecules such as *MYC*, *CDK2*, and *EZH2*, which play pivotal roles in muscle growth and differentiation. Alongside these growth factors, the regulation of key enzymes such as PRKACA and CALM3 further underscores the importance of cellular signaling in athletic adaptation. *PRKACA*, which encodes the catalytic subunit of protein kinase A (PKA), plays a pivotal role in various cellular processes, including metabolism, growth, and survival signaling. Studies have shown that *PRKACA* is upregulated in response to exercise, particularly in skeletal muscle, where it contributes to adaptations such as increased glycogen storage and enhanced muscle performance. The activation of PKA is crucial for the phosphorylation of downstream targets that regulate metabolic pathways, thereby facilitating the energy demands of highly trained individuals. Moreover, the upregulation of *PRKACA* has been associated with improved muscle hypertrophy and strength, as evidenced by its role in mediating the effects of resistance training on muscle growth factors [30].

On the other hand, *CALM3*, a member of the calmodulin family, is involved in calcium signaling and has been implicated in various physiological processes, including muscle contraction and neurotransmitter release. The expression of *CALM3* is also influenced by exercise, with evidence suggesting that its upregulation contributes to the enhanced calcium sensitivity of muscle fibers in trained individuals. This is particularly relevant as calcium signaling is essential for muscle contraction and overall muscle function, which are critical for athletic performance. Furthermore, CALM3's interaction with other signaling molecules underscores its role in mediating the effects of exercise on muscle metabolism and adaptation [31].

CALM3 plays a vital role in calcium signaling, impacting both muscle function and cardiovascular health. It helps regulate muscle excitation and cardiac rhythm, working alongside *CALM1* and *CALM2* to produce calmodulin proteins with tissue-specific expression patterns. In athletes, *CALM3* influences muscle adaptation and responsiveness, particularly in skeletal muscle during high-intensity activities. In this study, it was observed that there was a significant increase in *CALM3* expression in the athlete group compared to the non-athlete group. This showed that the presence of moderate and high intensity exercise affects the expression of several genes, one of which is *CALM3*. However, several studies have also shown that moderate to heavy activity can cause a decrease in *CALM3* gene expression. Previous studies have shown a decrease in *CALM3* gene expression in horse muscle four hours after exhausting treadmill exercise [32]. Otherwise, animal studies suggest exercise can alter *CALM3* expression, enhancing muscle metabolism and performance, hinting at similar benefits for human athletes [12,33].

In addition to muscle function, *CALM3* expressions also play a role in cardiac health, with potential implications for athletes' heart performance and safety. Variants of the *CALM3* gene have been associated with cardiac conditions such as long QT syndrome (LQTS), a disorder that can lead to arrhythmias under intense physical exertion [33,34]. Mutations or polymorphisms in the *CALM3* gene, such as the −34T>A variant in the promoter region, may influence its expression levels and, in turn, affect calcium signaling within the heart. Such genetic variations could alter cardiac responses during exercise, impacting an athlete's performance and posing potential health risks. The influence of *CALM3* polymorphisms on calcium homeostasis in the heart highlights the gene's broader significance in both athletic performance and cardiovascular health [35].

Finally, the impact of training on cAMP signaling pathways highlights the role of PRKACA in regulating muscle function and metabolic efficiency. This mechanism allows the body to respond efficiently to physical demands, highlighting the importance of PRKACA in exercise physiology and athletic performance [36]. *PRKACA* expression is known to adapt in response to training, which may enhance athletic performance over time. Studies have shown that endurance training can lead to changes in *PRKACA* expression in skeletal muscle, improving the muscle's oxidative capacity and overall energy efficiency [12]. This upregulation of energy-metabolizing genes, mediated by PKA, supports improved endurance and performance in athletes compared

to untrained individuals. Moreover, PRKACA's role varies with exercise type; for example, endurance training tends to increase the expression of genes related to mitochondrial function and energy metabolism, both of which are regulated by PKA pathways [37]. These adaptations highlight how PRKACA contributes to the physiological changes necessary for sustained athletic performance.

Beyond its role in muscle function and energy use, PRKACA may also impact athletic health. Moreover, genetic variants in the *PRKACA* gene could influence an athlete's response to training and recovery, as different polymorphisms may alter the efficiency of PKA signaling. Some of these genetic differences could predispose individuals to excel in specific sports by promoting faster muscle adaptation to training stimuli [33]. Furthermore, PKA signaling dysregulation has been linked to cardiovascular issues like hypertrophic cardiomyopathy, which poses potential risks for athletes under intense physical exertion. Thus, studying PRKACA not only sheds light on performance optimization but also underscores the need for awareness of potential health implications in athletic populations [35].

Conclusion

Using bioinformatics tools, this study successfully identified DEGs in resting skeletal muscle between sedentary individuals and highly trained athletes. A total of 426 genes were found to be significantly different, with 165 genes upregulated and 261 genes downregulated in trained individuals. These molecular adaptations reflect enhanced metabolic efficiency, improved mitochondrial function, and optimized muscle remodeling in response to long-term athletic training. The findings from GO and KEGG pathway enrichment analyses indicate significant upregulation of pathways related to muscle contraction, cardiovascular function, and DNA repair in trained individuals, further supporting their enhanced physical performance and metabolic health. The hub genes identified in this study, including *MYC*, *CALM3*, and *PRKACA*, play critical roles in muscle growth, differentiation, and adaptation. These results provide valuable insights into the molecular landscape of skeletal muscle in response to training regimens and offer potential targets for future research to improve athletic performance and muscle health.

Ethics approval

This study received ethical exemption from the Ethics Committee of Universitas Negeri Malang, as it met the standards of social and scientific value, equitable benefit-risk assessment, confidentiality, and informed consent, in accordance with WHO 2011 standards and CIOMS 2016 guidelines (Approval Number: No.23.08.7/UN32.14.2.8/LT/2024).

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author upon request.

Declaration of artificial intelligence use

This study used artificial intelligence (AI) tools and methodologies in the following capacities: data analysis, modeling and visualization. During data analysis, in this study, datasets were

sourced from multiple reputable biological databases, including the GEO database, GO, KEGG Pathway Database, and STRING database. These resources provided diverse and valuable biological data, which were meticulously selected and analyzed. Artificial Intelligence (AI) techniques, combined with advanced tools available in the R programming environment, were employed to identify complex patterns and extract meaningful insights, ensuring precision and efficiency throughout the data processing pipeline. For visualization purposes, the processed data were then visualized using AI-connected tools, such as Cytoscape (v3.10.2), the R package Limma (v3.54.2), clusterProfiler (v4.6.2), and pROC (v1.18.5), to reveal intricate biological relationships, functional annotations, and networks. These tools enabled the integration of complex datasets and facilitated the generation of clear, interpretable visualizations, enhancing our understanding of key patterns, pathways, and interactions within the data. We confirm that all AI-assisted processes were critically reviewed by the authors to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were solely made by the authors.

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