Research Article

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The potential mechanisms and regulation of myosin and energy metabolism in endurance training.

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ABSTRACT

Endurance training has great benefits for health and sports performances. However, the genes involved in endurance training and the trigged metabolic activities are still unclear. In this study, we provide basic insights into potential mechanisms of endurance training and clarify its regulatory effects on myosin and energy metabolism pathway by analyzing differentially expressed genes (DEGs). After analyzing the GSE242354 in the Gene Expression Omnibus (GEO) through bioinformatics techniques, 1325 DEGs were identified, including 200 up-regulated genes and 1125 down-regulated genes. Gene Ontology (G0) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis manifested that DEGs were mainly targeted at organic acid catabolic processes, etc. PPI network was constructed and explored in depth. Three important subnetworks and 14 hub differential genes such as GRWD1 were screened. Their crucial participation in RNA binding, preribosome and other processes were determined in enrichment analysis. The study of gastrocnemius muscle samples revealed that the expressions of MYH7B and MYH10 encoding actin heavy chain and MYL4 encoding light chain increased, indicating that endurance training could induce the enhanced expression of slow muscle fiber phenotype. In addition, FBP2, ALDOC and ENO1 regulated the glycolytic metabolic pathways and made exercises more energy-saving. The expression of ALDH6A1 was up-regulated after training, which affected the synthesis of long-chain fatty acids. This study identified some advisable mechanisms of endurance training and explained them to some extent. In addition, after long-term endurance training, energy metabolism will tend to be energy-saving and efficient in order to produce adaptation.

Keywords : *endurance training, bioinformatics analysis, DEGs, myosin, PPI network.*

INTRODUCTION

Endurance training is a pivotal component of physical and athletic regimens, offering significant advantages for health maintenance and performance enhancement. The multifaceted benefits of endurance exercise include inducing specific adaptations in the cardiovascular, respiratory, and neuromuscular systems (Nuuttila et al., 2022).

These adaptations facilitate oxygen transport to mitochondria and optimize muscle metabolism, enhancing athletes' endurance capabilities (Bizjak et al., 2020). This allows for sustained activity at a given intensity or the capacity to exercise at higher intensities (Jones and Carter, 2000). Further research has illuminated the physiological transformations post-training, such as the upregulation of mitochondrial protein content and enzymatic activity in skeletal muscles (Zoladz et

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al., 2022). Endurance training also bolsters cardiovascular function (Levine, 2014), modulates hormone levels (Stenqvist et al., 2020), and promotes redox balance (Rosa et al., 2020), skeletal muscle and angiogenic proteins increase (Hoier et al., 2020),etc. It has been demonstrated that the adaptive immune function by regulating the activity of various protein kinases in lymphocytes (Alack et al., 2020), inducing an increase in high-density lipoprotein synthesis, reducing the content of low-density lipoprotein and total cholesterol in the body to achieve healthy blood lipid levels (Tesema et al., 2019), inducing muscle hypertrophy and improving physical function (Yoshiko et al., 2019), eliminating inflammatory states, and reducing biomarkers of aging (Rosa et al., 2020).

However, despite extensive research, the mechanism of endurance training remains elusive (Ventura-Clapier et al., 2007). Current research has focused more on topics such as mitochondria, maximum oxygen uptake, and endurance performances (Baar, 2014; Yoshiko et al., 2019; Lang et al., 2021), while other aspects have not been extensively explored. The human body regulates metabolic pathways through genes to cope with external stimuli, and different exercise patterns activate and/or inhibit specific subsets of genes and cellular signaling pathways to regulate biological characterization (Hawley, 2009). Exploring metabolic pathways and other aspects may further explain the mechanism of endurance training. Bioinformatics analysis is a method of collecting and analyzing large amounts of omics data using new high-throughput molecular biotechnology, and then studying biomedical problems based on data research. In recent years, it has played an important role in multiple fields such as medicine and agriculture (Akl et al., 2014; Du et al., 2021; Zhou et al., 2024).

The aim of this study is to provide basic insights into some related mechanisms of endurance training by analyzing the differential genes before and after endurance training, and to elucidate the regulatory effects of multiple genes on myosin synthesis and energy metabolism pathways.

METHODS

Data sources

The workflow of the study is provided in Fig.1. Briefly, the dataset GSE242354 were obtained from an open gene expression comprehensive database GEO submitted by Barrett et al. (2013). This dataset includes high-throughput sequencing analysis data of expression profiles from various tissue samples of young adult Norwegian mice after endurance training and healthy control Norwegian mice. The analysis platform used in this dataset is GPL25947, which includes a total of 915 samples. This study selected 20 whole blood samples, of which 10 were from Norwegian mice in the intervention group who received endurance training for 8 weeks, and 10 were from the control group.

Figure 1

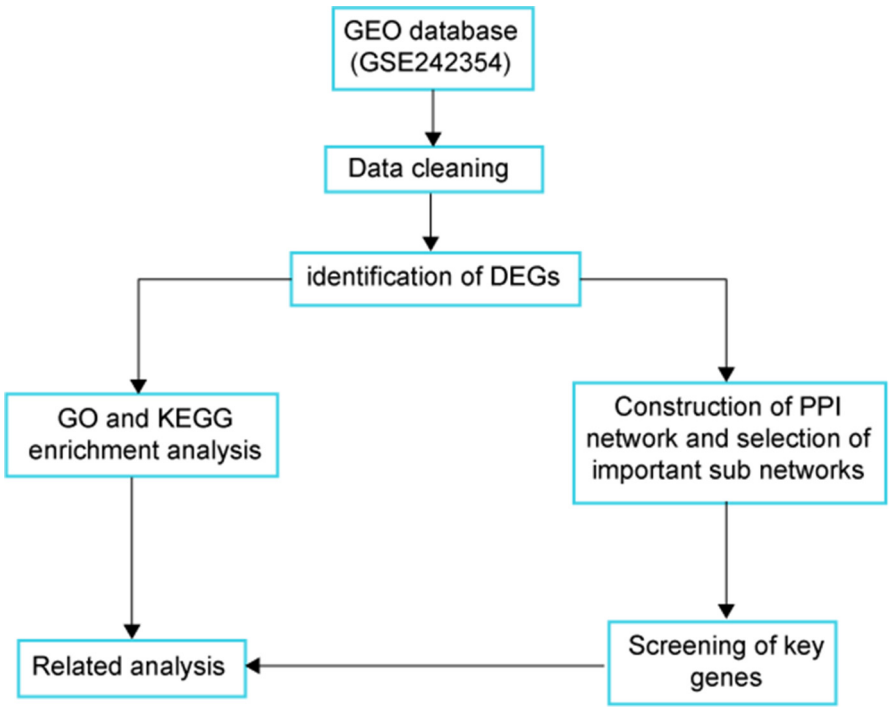


Fig. 1. The workflow diagram of this study

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Data cleaning and organization

Ensembl is a database containing genomic gene information of vertebrates, which can be used to obtain gene annotation information, predict corresponding functions, etc. (Martin et al., 2023). In RStudio (version 2023.12.0.369), use the officially provided biomaRt package (version 2.58.0) by Ensembl to convert the Ensembl id in the obtained expression matrix into human homologous genes, and exclude genes with 0 expression levels in 20 samples, retaining the remaining genes for further analysis. For duplicated genes, take the average value and retain it.

Screening of differentially expressed genes (DEGs)

In RStudio, the data in the expression matrix was first subjected to 'log2' transformation to complete normalization. Then, Limma package (version 3.58.1) was used to screen differentially expressed genes (DEGs) in the whole blood of Norwegian mice and the control group after 8 weeks of endurance training. When log | FC | > 0.58 (i.e., fold change > 1.5), $p < 0.05$, the gene is identified as DEGs. Visualize the obtained DEGs to obtain a volcano map and draw a heat map.

Functional enrichment analysis

To investigate the potential role of the obtained DEGs, we conducted GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) functional enrichment analysis (Kanehisa et al., 2017; Thomas et al., 2022). GO enrichment analysis obtains biological characteristic results by annotating genes or their products, identifying gene chip data. KEGG analysis is a commonly used method for gene pathway analysis, which can annotate and enrich genes on signaling pathways for research. This study used cluster profiler (version 4.8.2) in RStudio to analyze DEGs, and $p < 0.05$ was considered statistically significant. Plot the obtained results for visual exploration of their biological functions.

Construction of protein-protein interaction network (PPI) and screening of important sub networks

Protein Protein Interaction Networks (PPI) are composed of proteins that participate in various life processes such as biological signal transduction, gene expression regulation, energy and substance metabolism, and cell cycle regulation through their interactions with each other. Build a PPI network for DEGs using the STRING database (Szklarczyk et al., 2023). Choose confidence as the basis for building, set the minimum required interaction score (confidence) to 0.700, remove discrete proteins, and generate a PPI network. Beautify the generated PPI using Cytoscape software (version 3.10.1) and filter out important downstream subnetworks using the MCODE plugin (Bader and Hogue, 2003).

Screening of PPI Network Hub Genes

We use the Cytohubba plugin (Chin et al., 2014) in Cytoscape to screen hub genes in the network. Based on the attribute ranking of nodes in the PPI network, five commonly used algorithms (MCC, DMNC, MNC, Degree, EPC) were used to evaluate and select hub genes. Then, the top 50 genes of each algorithm are intersected and visualized using the upsetR package (version 1.4.0) to identify the genes in the intersection as hub genes.Use the Gene mania plugin (version 3.5.3) to predict potential relationship networks related to hub genes, followed by GO enrichment analysis to speculate possible changes caused by changes in hub genes.

Analysis of Myosin and Partial Energy Supply Metabolic Pathways

To explore the changes in important pathways related to exercise, we enriched the expression matrix information in gastrocnemius muscle samples in KEGG and selected myosin synthesis and energy supply metabolism pathways that are highly related to exercise and significantly enriched to analyze the changes.

RESULTS

Screening of differentially expressed genes (DEGs)

After comparing and analyzing the endurance training group and the control group, a total of 1325 DEGs were obtained, of which 200 were up-regulated genes and 1125 were down-regulated genes, and we visualized through volcano and heat-maps (Fig. 2).

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Figure 2

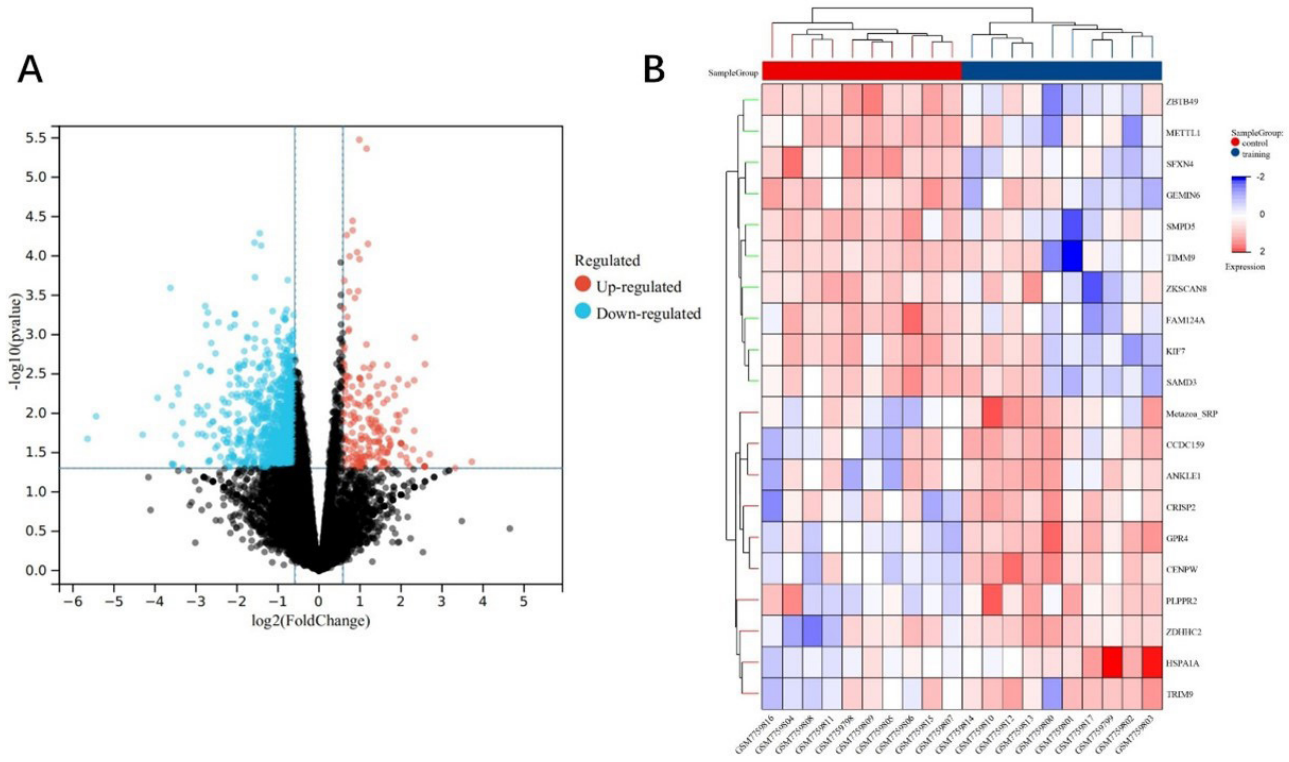


Fig. 2. DEGs of endurance training group and control group. (A) Volcano map of DEGS. Among them, red and blue represent heat-maps of up-regulated and down-regulated genes (B) Heat map of the first 20 DEGs.

Functional enrichment analysis results of DEGs

The GO enrichment analysis of DEGs manifested that organic acid catalytic process, carboxylic acid catalytic process, ncRNA processing, small molecule catalytic process, and tRNA processing were the five most significantly enriched biological processes. Mitochondrial matrix, spin, collagen containing extracellular matrix, DNA-directed RNA polymerase complex, and STAGA complex are the top five cell components with the most significant expression differences. Modified amino acid binding, acyl-CoA binding, sulfur compound binding, tRNA binding, and aldo-keto reductase (NADP) activity are the top five most significantly altered molecular functions (Fig. 3A, 3B, 3C). KEGG pathway analysis showed that Primary bill acid biosynthesis, Cytosolid DNA sensing pathway, Alanine, aspartate and glucose metabolism, Hematopoietic cell lineage, Protein digestion and absorption were the top five enrichment pathways (Fig. 3D).

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Figure 3

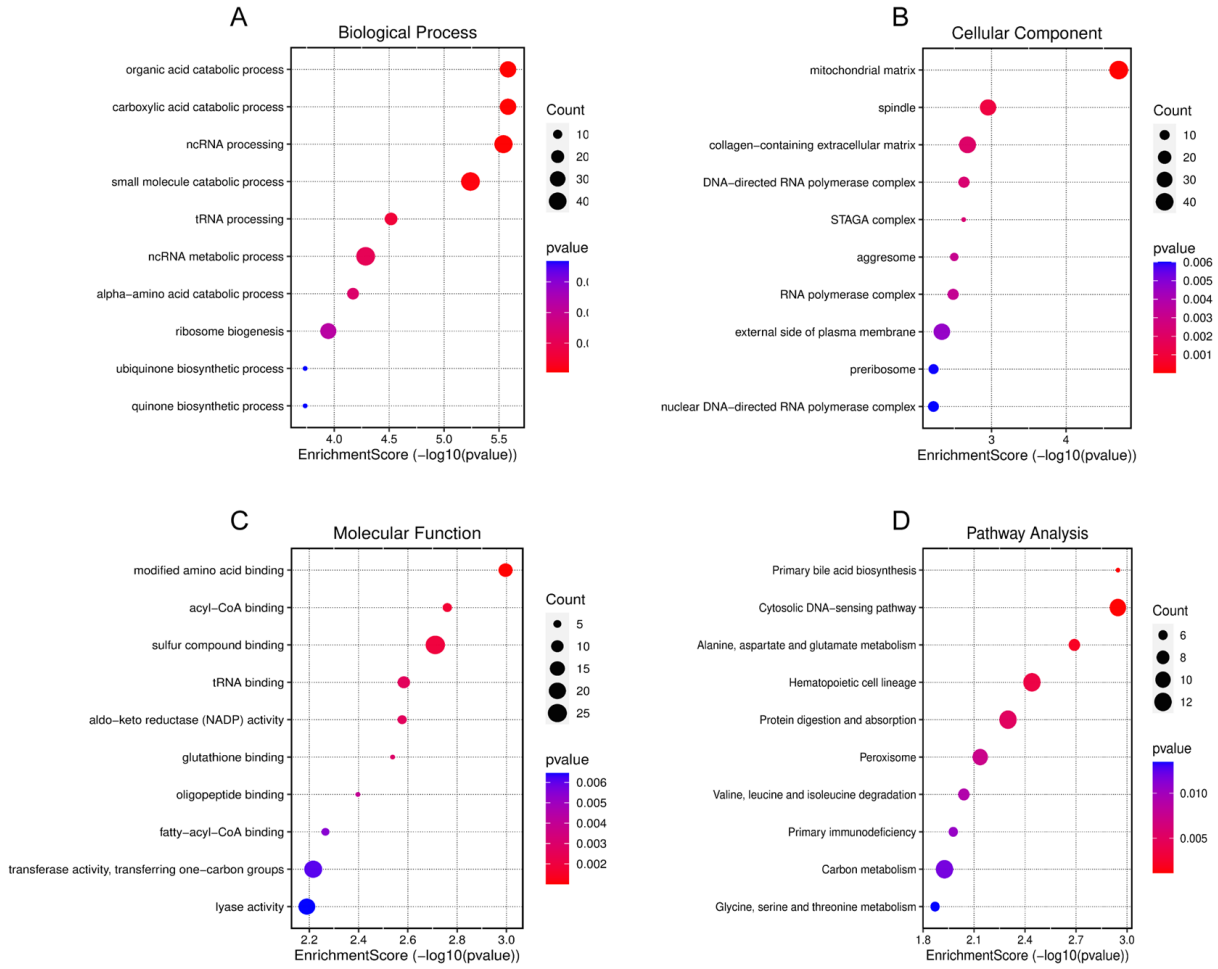


Fig. 3. Results of DEGs' GO and KEGG enrichment analysis. A-C show the top biological processes, cellular components, and molecular functions in GO analysis. D shows the results of KEGG pathway enrichment.

Construction of protein-protein interaction network (PPI) and screening of important sub networks

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We performed online analysis of DEGs using the string database. We obtained the PPI network and we used Cytoscape for beautification. This network (Fig. 4A) contained 420 nodes and 2246 edges. The top three important modules were identified using the MCODE plugin in Cytoscape. Module One (Fig. 4C) contained 28 nodes and 348 edges, with an analysis score of 12.889. Module Two (Fig. 4B) contained 13 nodes and 132 edges, with a score of 11.000. Module Three (Fig. 4D) contained 10 nodes and 54 edges, with a score of 6.000.

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Figure 4

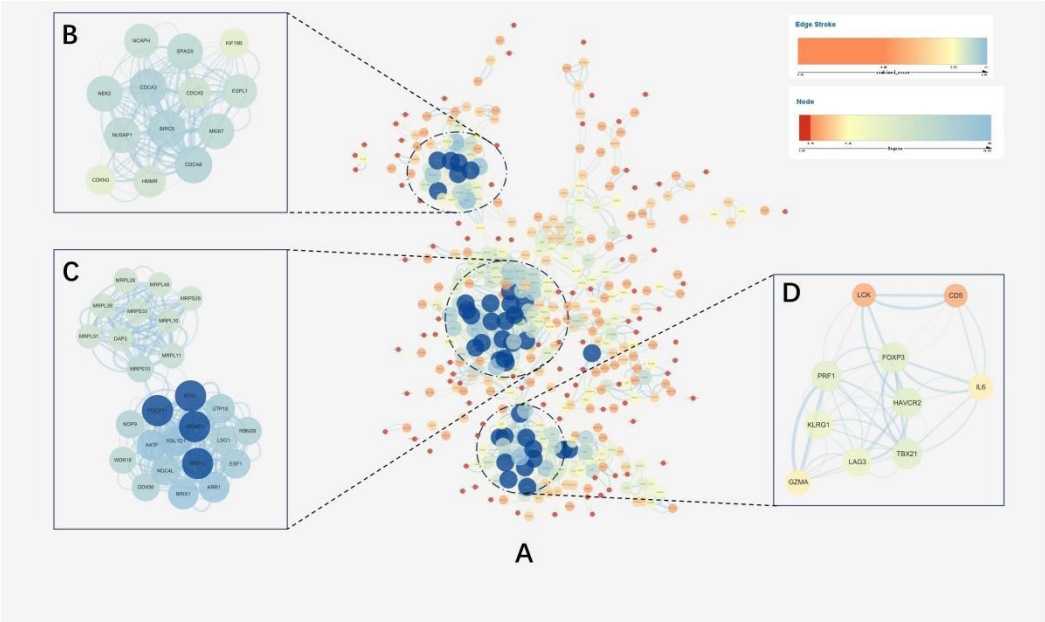


Fig. 4. Overview diagram of PPI network. A, the outline of the entire PPI network; B, C, and D showed three important sub network modules in the network respectively.

Screening of hub genes in PPI network

In Cytoscape, the Cytohubba plugin was used to calculate the criticality ranking of genes using five algorithms. The upsetR package was used to find the intersection of the top fifty genes ranked by the five algorithms in R language (as shown in upset Fig. 5), and 14 hub genes (GRWD1 RRP12 RSL1D1 BRIX1 KRR1 ESF1 AATF UTP18 NOC4L RBM28 LSG1 DDX56 NOP9 WDR18) were obtained from the PPI network.

Figure 5

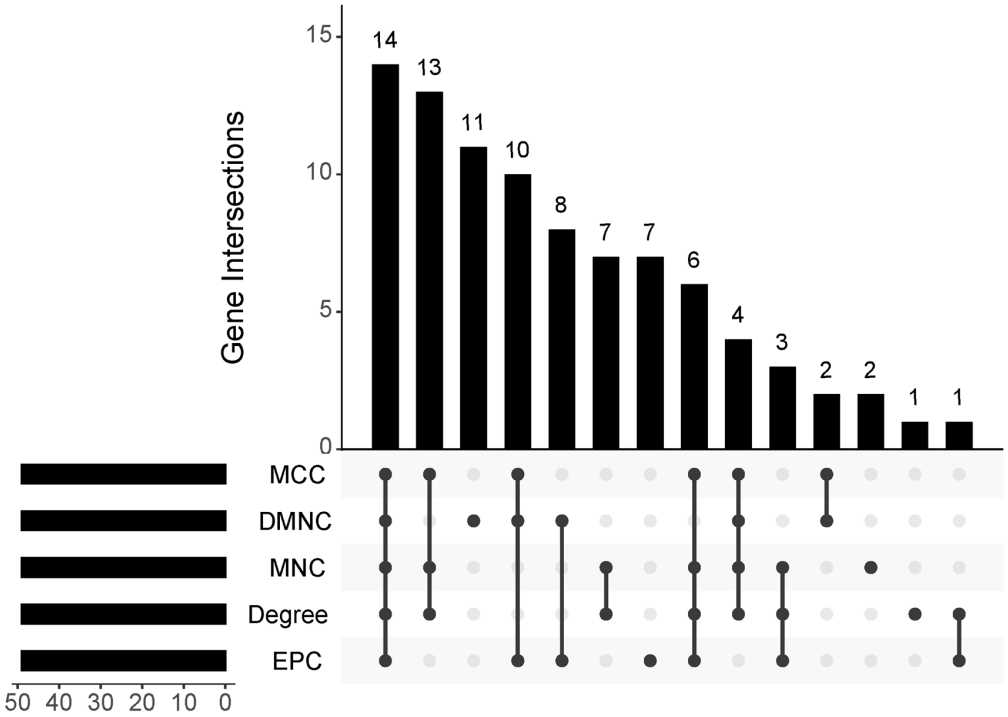


Fig. 5. Upset graph of hub genes obtained based on five algorithms. The Figure shows the process of performing intersection processing on the key genes selected by five commonly used cytoHubba algorithms. A total of 14 hub genes were obtained.

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Use the Gene mania plugin to link 14 key genes with the most likely related genes to form a hypothetical PPI network containing 34 genes (Fig. 6A), and perform GO enrichment analysis to infer possible changes caused by changes in hub genes. The results showed that these 34 genes were mainly clustered in 4 molecular functions, 31 biological processes, and 24 cellular components. After endurance training, the 14 genes that play a crucial role in the protein interaction network may induce significant RNA binding activity, preribosomes, and ribosomes biogenesis in the body (Fig. 6B, 6C, 6D).

Figure 6

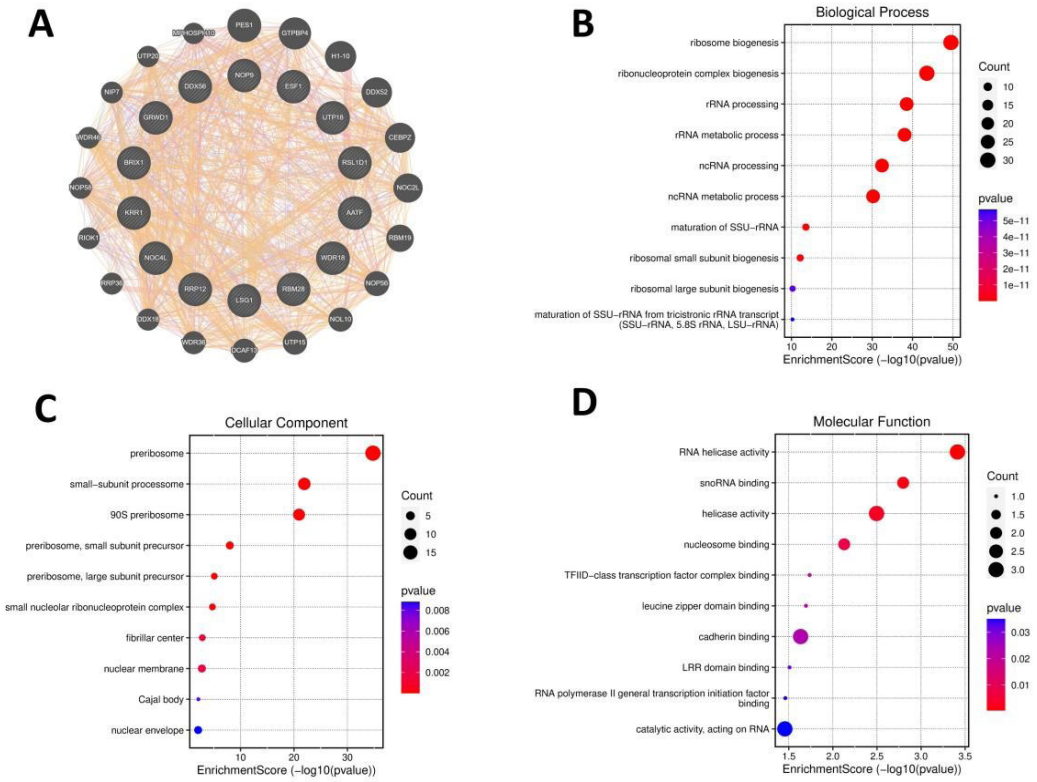


Fig. 6. The PPI network predicted by Gene Mania and the results of enrichment analysis. A, the protein interaction network of the 14 hub genes. B, enriched biological processes; C, molecular processes; D, cellular components.

Analysis of myosin synthesis and energy supply metabolic pathways

In the process of functional enrichment, we studied gastrocnemius muscle samples and specifically explored myosin synthesis and some energy supply metabolic pathways related to exercise training. We found that MYH7B and MYH10, which encode the heavy chains of myosin, and MYL4, which encode the light chain, were all up-regulated, indicating that endurance training can induce enhanced phenotype expression of slow muscle fibers, leading to increased endurance and less fatigue in the body (Fig. 7).

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Figure 7

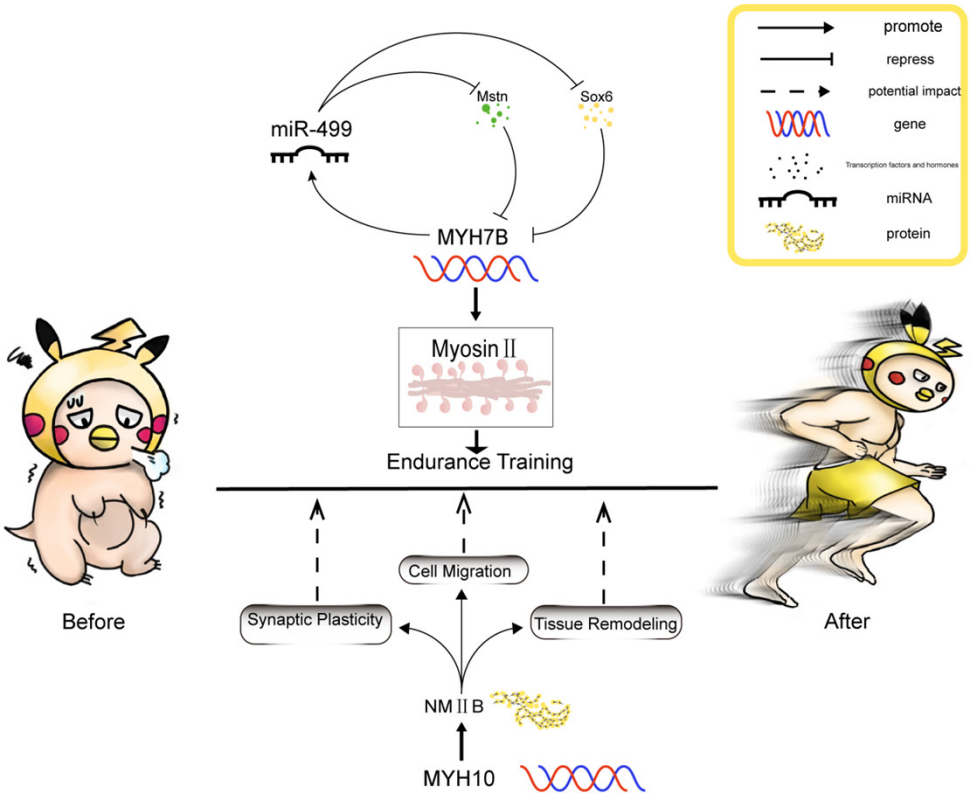


Fig. 7. Schematic diagram of the process of regulating myosin. The biological process changes related to myosin regulatory genes (MYH7B, MYH10) before and after endurance training.

Glycolysis is one of the main energy supply systems in the body during exercises. We found that endurance training significantly changes the FBP2 (up-regulated), ALDOC (down-regulated), and ENO1 (down-regulated), which might indicate that endurance exercise regulates the glycolytic metabolic pathway and make exercise more energy-efficient (Fig. 8).

Figure 8

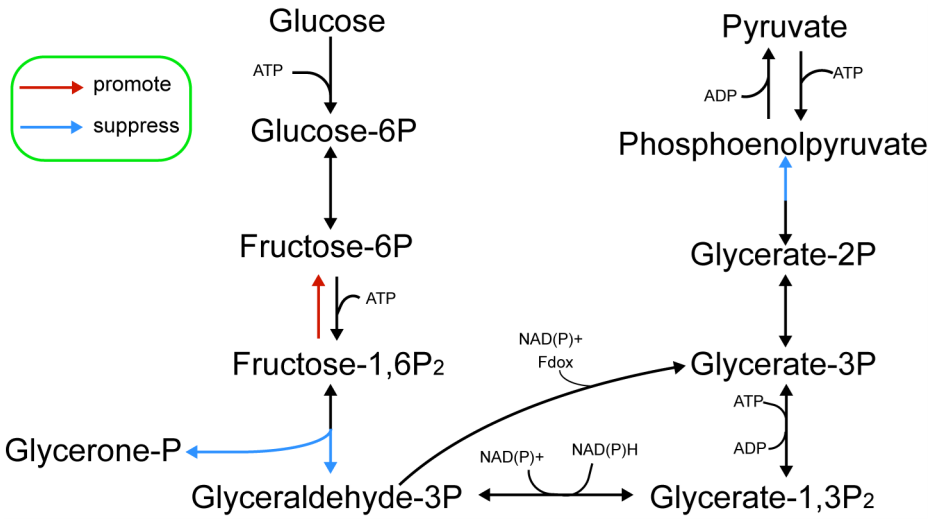


Fig. 8. The changes in glycolytic metabolic pathways after endurance training.

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DISCUSSION

Endurance training positively impacts athletic performance and maintains physical health. Despite this, the current understanding of endurance training remains superficial. Gaining insight into the underlying mechanisms is essential for a deeper appreciation of the benefits of exercise on the body and for the informed design and execution of training programs and physical activities. In this study, we obtained 1325 DEGs before and after endurance training through limma differential analysis, and conducted functional enrichment analysis.

Myosin plays an important role in skeletal muscle contraction. Skeletal muscle fibers can be divided into four subtypes of myosin heavy chain (MyHC) (Type I, IIA, IIB, and IID/X). The subtypes of myosin can be divided into two types of muscle fibers: one is slow muscle fibers with strong red oxidation ability and anti-fatigue ability and the other is white, another is the fast muscle fibers that are more prone to fatigue (Bassel-Duby and Olson, 2006; Schiaffino and Reggiani, 2011). MYH7B is a gene that encodes the heavy chain of myosin II. The heavy chain subunit it encodes is a type of slow myosin, which can act as an engine for actin during contractile activity. It converts the chemical energy obtained by catalyzing ATP hydrolysis into the mechanical force required for macroscopic muscle contraction and interacts with actin to complete contractile activity. It mainly plays a role in slow and energy-saving life movements (Lee et al., 2023). Meanwhile, the specific microRNA network in skeletal muscle can coordinate muscle fiber types and play a core role in regulating skeletal muscle plasticity (van Rooij et al., 2009). The miR-499 contained in MYH7B is involved in the designation of muscle fiber types in skeletal muscle. Its targeted series of transcriptional inhibitors inhibit the inhibitory genes of slow muscle fibers (such as Sox6, Mstn, etc.), activate the slow muscle fiber generation program, and generate a positive feedback loop, thereby promoting the expression of the slow muscle fiber phenotype (van Rooij et al., 2009; McCarthy, 2011). Therefore, after receiving endurance training, up-regulation of MYH7B gene expression in the gastrocnemius muscle can, to some extent, increase the synthesis of myosin II heavy chain, increase ATP hydrolysis efficiency during muscle contraction, and enhance energy supply efficiency. At the same time, it enhances the phenotype expression of slow muscle fibers, making the body adapt specifically to the activities required for long-term endurance training, increasing endurance and reducing fatigue.

Non-muscle myosin II (NMII) is crucial for maintaining the structure and function of cells. This mechanism plays an important role in processes such as cell migration (Vicente-Manzanares et al., 2009a; Newell-Litwa et al., 2015). Another MYH10 gene regulated in gastrocnemius muscle samples encodes non-muscle myosin IIB (NMIIB). NMIIB is an important subtype of NMII that participates in important physiological activities such as synaptic plasticity. It has the characteristics of NMII, which can generate contraction force within cells through interaction with actin filaments, facilitating the formation of actin filaments. These filaments interact with adhesive complexes, generating deformation forces that affect the cell membrane and its substrate (Vicente-Manzanares et al., 2009b; Murrell et al., 2015). In migrating cells, NMIIB is typically located at the posterior end of the cell and plays a crucial role in cell motility (Murrell et al., 2015). The up-regulation of MYH10 expression in gastrocnemius muscle samples may indicate the presence of enhanced processes such as cell migration and tissue remodeling which are guided by endurance training. MYL4 is a gene encoding myosin light chain, presenting in embryonic muscles and atria (Vicente-Manzanares et al., 2008; Vicente-Manzanares et al., 2009b). The encoded light chain plays a regulatory role as part of myosin, helping to control the strength and speed of muscle contraction (Schiaffino and Reggiani, 2011). Due to the lack of sufficient evidence to support the expression of MYL4 gene in adult skeletal muscles, a possible explanation for the presence of MYL4 gene expression in the samples is that endurance exercise promotes MYL4 gene expression to correct possible autophagy dysfunction and lysosomal function in the body (Schiaffino et al., 2015).

The energy supply system is crucial for sports. Our research findings suggest that there may be inhibitory effects on the glycolytic energy supply pathway after endurance training, and the body becomes more dependent on other energy supply pathways (Fig. 8). Among them, FBP2 (up-regulated) may play a key role in this process. FBP2 encodes a gluconeogenic regulatory enzyme, which is able to catalyze the hydrolysis of fructose 1,6-bisphosphate to fructose 6-phosphate (Wang et al., 2019; Zhong et al., 2023). The FBP2 gene is significantly up-regulated in the sample, leading to an increase in this enzyme's activity, enhancing the one-step retrograde response during glycolysis, thereby inhibiting the production of fructose 1,6-bisphosphate and directly reducing the rate of glycolysis (Huangyang et al., 2020).

In addition, during the exploration of this pathway, we found that the expression of ALDOC and ENO1 genes was down-regulated to a certain extent. Due to the lack of sufficient evidence supporting that the changes in genes regulating the subsequent reactions are caused by the endurance training,rather than the regulatory changes which could be considered as the responses to the weakening of body's forward reaction caused by the decrease of reaction substrate led by the up-regulation of FBP2, our results cannot prove that endurance training has a direct effect on down-regulation of ALDOC and ENO1 expression. However, it is certain that down-regulation of these two genes will further reduce the rate of

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glycolysis: the ALDOC gene encodes a fructose-biphosphate aldolase that plays a role in glycolysis, catalyzing the reversible aldol cleavage of fructose-1,6-biphosphate to glyceraldehyde 3-phosphate and glycerone phosphate (Gerhard et al., 2004; Li et al., 2013). Down-regulation of the ALDOC gene leads to a decrease in the production of this enzyme, reducing the production of glycerone phosphate and glyceraldehyde 3-phosphate, and inhibiting subsequent reactions. ENO1 encoding α- Enolase, which catalyzes the reaction of 2-Phospho-D-glycerate to produce Phosphoenolpyruvate (Izraely et al., 2021),decreases expression and reduces the production of Phosphoenolpyruvate. For the entire pathway, our study supports that up-regulation of the FBP2 gene induced by endurance training leads to a decrease in the rate of glycolytic energy supply. This may mean that the body has reduced the proportion of glycolytic energy supply to adapt to regular endurance training and has begun to rely more on other more energy-efficient energy supply systems. However, it has not yet been possible to believe that endurance training can directly regulate the expression changes of ALDOC and ENO1, and further evidence is needed to support this.

Among other energy metabolism pathways, acyl-CoA metabolism is a group of biochemical metabolic pathways associated with acyl-CoA, which involves binding long-chain fatty acids and other compounds to CoA (Fujita et al., 2014). A portion of acetyl-CoA obtained from glycolysis will react and generate malonyl-CoA, which enters this metabolic pathway for energy production, lipid synthesis, and other biological processes. These metabolic pathways play a crucial role within cells and are related to the synthesis, decomposition, oxidation, and transportation of fatty acids (Grevengoed et al., 2014; Song et al., 2014). We found that the gene ALDH6A1, which encodes a regulatory enzyme for this metabolic pathway, was up-regulated after training. This enzyme catalyzes the production of acetyl-CoA from 3-oxopropionate in the Acyl-CoA metabolis pathway, reducing the amount of acetyl-CoA entering the pathway and simultaneously affecting the synthesis of long-chain fatty acids. The changes that occur in the body may be to allow more acetyl-CoA to enter the tricarboxylic acid cycle for complete oxidation and release more energy. Overall, after long-term endurance training, the body's energy metabolism tends to become more energy-efficient and efficient in order to adapt. The inhibitory regulation of glycolysis means that the body can save more glucose to ensure the persistence of exercise, and the corresponding energy gap may be compensated for through more energy-efficient metabolic pathways.

CONCLUSIONS

This study identified the mechanisms of endurance training and explained the energy-saving adaptation of muscle fiber type regulation and energy supply system. The endurance training involved complex physiological mechanisms and involved many interactive processes. By delving deeper into these mechanisms, we can better explain the principles of endurance training, guide sports and physical exercise, and maintain physical health.

Acknowledgement

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Not applicable.

Contributions

Wang conceived and designed this research, and completed the writing of the paper. Cui completed the bioinformatics analysis and schematic diagram production. Cao completed the illustration related work for the paper. Bian and Ming evaluated and revised the manuscript. All authors have read and approved the final manuscript.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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