Original Article

Muscle genomics and aerobic training

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ABSTRACT

The performance in physical activity is determined not only by physiological processes such as age, body composition, gender and degree of training, but also by the genomics and even epigenetic events occurring during the training programs. In this context, using bioinformatics resources, we aimed to analyse the expression of genes associated with muscle function in vastus lateral samples. We used data from DNA microarray experiments reported in NCBI's GEO DataSet database under the series number GSE117070. Differential expression was calculated using the Z-ratio equation. We also used the software Cytoscape 3.6 to build a protein-protein interaction network with over-expressed genes. We found that seven genes out of the 397 genes analysed in the 41 individuals subjected to aerobic exercise with an increase in training intensity through the percentage of VO_{2max}, were over-expressed based on the statistical approach. The Protein-Protein Interaction (PPI) network showed 477 nodes, two connected components, 17 multi-edge node pairs and an average number of neighbours of 2.092. The node with the highest number of interactions was TPM1 with 150. GO categories of biological processes most relevant of the network included indispensable processes for muscle function and contraction such as polymerization of actin filaments and ATP synthesis from electron transport chain.
Keywords: Aerobic training; Muscle; DNA microarray; Differential expression; Network.

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INTRODUCTION

The type of exercise and the training load during the exercise to which the athlete is subjected modifies muscle contraction and the VO_{2max} This measurement is defined as the maximum amount of O_2 that the body is able to absorb, transport and is consumed per unit of time (Wasserman, et al.,). VO_{2max} is regulated by metabolic changes and by a wide variety of protein isoforms at the fibre level (Di Prampero, et al., 2015) (Samozino, et al., 2016), caused by functional regulation of the genome. This is determined by variations in DNA sequences, methylation of the promoter and coding regions of genes, topological changes in the structure of chromatin, homeotic regulation of genes and by small RNA molecules, also known as microRNAs in skeletal muscle (Liu, et al., 2010) (Pilegaard, et al., 2000).

The physiological processes that determine the performance in physical activity or sport are the following: age, body composition, gender, degree of training. However, there are global genomic processes that participate in the complexity of the manifestations of performance for people who perform a monitoring in the sports load, these manifestations are mediated by alterations in the number of copies of functional and non-traditional genomic elements. Associated with this, epigenetic mechanisms could perform transcriptional modifications of the skeletal muscle through the induction of gene expression changes that do not necessarily alter the nucleotide sequence, but in a small percentage can generate some polymorphism.

Currently, experiments with DNA microarrays allow examining transcriptional changes in the skeletal muscle with a previous gene characterization based on age, gender, strength and muscular resistance (Rowlands, et al., 2011) (Timmons, et al., 2010). It is important to note that the signalling histones, the binding of the transcription factor and the chemical modifications of the DNA can actively influence or passively reflect the musculoskeletal expression programs, which can affect the activity of the isoform that performs the contraction through molecular signalling events (Rockman & Kruglyak, 2006) (Visscher, et al., 2017). Associated with this, speed exercises allow a percentage development of fast-twitch muscle fibres and resistance or aerobic exercises allow the development of a high percentage of slow-twitch muscle fibres (Saltin, et al., 1977) (Trappe, et al., 2015).

Understanding these changes will improve the development of sports planning that will not only take into account the phenotypic change that, in the case of aerobic exercise, would primarily be oxygen consumption, but would also make it possible to determine the markers of a type of fibre or other metabolic modifications in the tissue, reflected by the muscular transcriptome. In this context, we aimed to analyse the gene expression of genes associated with muscle function in vastus lateral samples, by using data from DNA microarray experiments recorded in NCBI's GEO DataSet database (https://www.ncbi.nlm.nih.gov/gds/), GSE117070. Differential expression was calculated using the Z ratio. We found overexpressed and sub expressed genes associated with muscle function after a 20-week training incrementally.

MATERIALS AND METHODS

To perform the bioinformatic analysis of the present study, we selected 397 genes associated with muscle function, previously reported in the Gene Entrez of the NCBI database. (https://www.ncbi.nlm.nih.gov/gene), which are described in Supplementary Table 1. We used the transformed expression values of log2 intensity of a DNA microarray experiment whose registration code and free access in the GEO database was GSE117070 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE117070) (Bouchard, 1995). The selected microarray experiment included gene expression data from more than 43,797 probes from 82 samples of the quadriceps muscle (vastus lateralis) from 90 Caucasians and 40 African American, with a

range of age between 17 and 65 years old, the selected microarray experiment included gene expression data from more than 43,797 probes from 82 samples of the quadriceps muscle (vastus lateralis) from 90 Caucasians and 40 African American, with a range of age between 17 and 65 years old, for the category of "*sons*" between 17 and 40 years old and for the category of "*parents*" between 40 and 65 years old. They could not have done any physical activity in the last 3 months before starting the study, nor have had any chronic non-contagious disease. The entire population was evaluated before and after the 20-week aerobic exercise program. There was no group control as in the usual studies, because they were the same cases at the beginning of the 20-week program and their own controls after the 20 weeks. Regarding the gene expression profiles, they were determined from the muscle biopsies, to subsequently perform Affymetrix microarray analysis, at the beginning and at the end of the 20-week aerobic exercise program.

This was developed as follows: the first two weeks at 55% of VO_{2max}, each week 3 sessions of 30 minutes each, the next two weeks at 55% of VO_{2max}, each week 3 sessions of 35 minutes each. The following two weeks at 65% of VO_{2max}, each week 3 sessions of 35 minutes each, the next two weeks at 65% of VO_{2max}, each week 3 sessions of 40 minutes each. The next two weeks at 70% of VO_{2max}, each week 3 sessions of 40 minutes each, the next two weeks at 70% of VO_{2max}, each week 3 sessions of 45 minutes each. The next two weeks at 75% of VO_{2max}, each week 3 sessions of 45 minutes each and at the end, 6 weeks at 75% of VO_{2max}, each week 3 sessions of 50 minutes each. All this was done on a cycle ergometer with continuous monitoring of the heart rate (Bouchard, 1995). However, for the present study, we decided to study the genes associated with muscle function before and after the application of the training program.

Differential gene expression quantification

Raw intensity log2 data for each experiment was used for the calculation of Z-score (Cheadle, et al., 2003). Z-scores of the protein coding genes analysed, were calculated according to the Equation 1:

$$Z - score = \frac{(Log\ intensity\ of\ G - mean \log intensity\ G \dots Gn)}{Standard\ Deviation \log G \dots Gn}$$

Equation 1. Z-score formula.

All values of the Z score were normalized on a linear scale $-3.0 \leq 0 \geq +3.0$ (two-tailed p-value <.001). From the data of the z-score, we calculate the average values per gene for pre-training and post-training. These data were used to calculate the Z ratio (Equation 2) with a measure to estimate the differential gene expression; genes with values higher than 1.96 are considered overexpressed (Cheadle, et al., 2003).

$$Z - ratio = \frac{[(Z - score_{G1ave})Pos - training - (Z - score_{G1ave})Pre - training]}{SD\ of\ Z\ score\ differences G1 \dots Gn}$$

Equation 2. Z-ratio formula.

The Z-test, allows to determine by means of calculations the gene expression, maximizing the power of the replicas taking into account the variance of gene to gene:

$$Z-ratio = \frac{[(Z-score_{G1ave})Pos-training-(Z-score_{G1ave})Pre-training]}{\sqrt{\frac{\sigma}{n1}+\frac{\sigma}{n1}}}$$

Equation 3. Z-test formula.

Where G1 represents the average Z score for gene being tested under experimental conditions, for this study, post-training versus pre-training. The repeated hybridization intensity measurements (expressed as Z scores) are mean difference corrected by the SE for the difference between means where σ is the SD, for either condition 1 or condition 2, and n equals the number of repeated measurements for either condition 1 or condition 2.

Interaction network
We used the software Cytoscape 3.6 to build a network of expression and interaction entering a matrix created with the information of physical protein interactions placed in the BioGRID database. Ontological categories were extracted from the network in the Cytoscape 3.2 program using the plugin BiNGO, which establishes a remote connection to the database Gene Ontology, reporting the most important biological processes of the network, with their own p-value (Bonferroni correction). For the present study, we chose 10 processes with the most significant p-values.

Statistical analysis
Analyses were performed to compare the media values of the Z score between post-trained and pre-trained. The Wilcoxon signed-rank /Two Tailed was used to calculate the differences between the media of two samples. In all cases we use an alpha .05 to test the importance of H_0. On the other hand, to calculate the statistical differences in the log2 values of Post-trained and Pre-trained, we applied the t test for two paired samples / two-tailed test with an alpha of .05.

Principal component analysis (PCA) was employed as a computational procedure for the classification of multiclass gene expression in muscle biopsy samples from individuals in post-trained and pre-trained conditions. All analyses were performed in the SPSS program, version 25.

RESULTS

Table 1. Over-expressed genes associated with muscle functions.

Gene ID	Symbol	Z-score Post	Z-score Pre	Z-ratio	p-value*
7168	TPM1	0.10790368	-0.10352638	284.064.636	< .00001
1282	COL4A1	224.952.369	205.107.125	266.628.685	< .00001
4617	MYF5	-0.0230669	-0.21585979	259.024.856	< .00001
4478	MSN	-0.2643089	-0.44033643	236.499.942	< .00001
9639	ARHGEF1	-0.14934508	-0.312698	219.471.096	< .00001
7052	TGM2	-0.48019236	-0.64051953	215.405.883	< .00001
91746	YTHDC1	-0.14333736	-0.29550284	204.440.332	< .00001

(*). Is statistically significant p-value.

Supplementary Table 1. Over-expressed genes associated with muscle function.

Gene ID	Symbol	Name	Locus	Function
7168	TPM1	Tropomyosin 1	15q22.2	This gene is a member of the tropomyosin family of highly conserved, widely distributed actin-binding proteins involved in the contractile system of striated and smooth muscles and the cytoskeleton of non-muscle cells. Tropomyosin is composed of two alpha-helical chains arranged as a coiled-coil. It is polymerized end to end along the two grooves of actin filaments and provides stability to the filaments.
1282	COL4A1	Collagen type IV alpha 1 chain	13q34	Collagen alpha-1(IV) chain; Type IV collagen is the major structural component of glomerular basement membranes (GBM), forming a 'chicken-wire' meshwork together with laminins, proteoglycans and entactin/nidogen.
4617	MYF5	Myogenic factor 5	12q21.31	Acts as a transcriptional activator that promotes transcription of muscle-specific target genes and plays a role in muscle differentiation. Together with MYOG and MYOD1, co-occupies muscle-specific gene promoter core region during myogenesis. Induces fibroblasts to differentiate into myoblasts. Probable sequence specific DNA-binding protein; Basic helix-loop-helix proteins.
4478	MSN	Moesin	Xq12	Probably involved in connections of major cytoskeletal structures to the plasma membrane. May inhibit herpes simplex virus 1 infection at an early stage. Plays a role in regulating the proliferation, migration, and adhesion of human lymphoid cells and participates in immunologic synapse formation; FERM domain containing.
9639	ARHGEF10	Rho guanine nucleotide exchange factor 10	8p23.3	May play a role in developmental myelination of peripheral nerves; Rho guanine nucleotide exchange factors.
7052	TGM2	Transglutaminase 2	20q11.23	Catalyses the cross-linking of proteins and the conjugation of polyamines to proteins; Transglutaminases.
91746	YTHDC1	YTH domain containing 1	4q13.2	Regulator of alternative splicing that specifically recognizes and binds N6-methyladenosine (m6A)-containing RNAs. M6A is a modification present at internal sites of mRNAs and some non- coding RNAs and plays a role in the efficiency of mRNA splicing, processing and stability. Acts as a key regulator of exon-inclusion or exon-skipping during alternative splicing via interaction with mRNA splicing factors SRSF3 and SRSF10.

Out of 397 genes that were analysed, seven of them were significantly overexpressed in the vastus lateralis muscle samples analysed. They corresponded to: Tropomyosin 1 (TPM1), Collagen type IV alpha 1 chain (COL4A1), Myogenic factor 5 (MYF5), Moesin (MSN), Rho Guanine Nucleotide Exchange Factor 10 (ARHGEF10), Transglutaminase 2 (TGM2) and YTH Domain Containing 1 (YTHDC1) were overexpressed (Table 1). That is, 1.9% of the analysed genes explained the behaviour of VO_2 in relation to skeletal muscle activity, for that population.

The Protein-Protein Interaction (PPI) network made with the seven overexpressed genes associated with muscle function found in the previous analysis showed a total of 477 nodes, two connected components, 17 multi-edge node pairs and an average number of neighbours of 2.092 (Figure 1). The node with the highest number of interactions was TPM1 with 150, followed closely by MSN with 142. GO categories of biological processes most relevant of the network (Table 2) include indispensable processes for muscle function and contraction such as polymerization of actin filaments (p-value 2.57E-14) and ATP synthesis from electron transport chain (p-value 1.58E-13). According to the co-expression network made in GENEMANÍA, the relevant functions associated were muscle contraction and extracellular matrix organization.
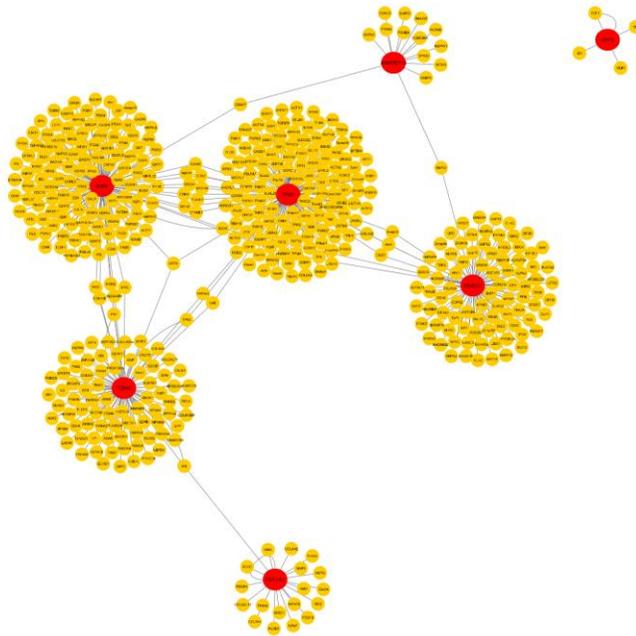

Figure 1. Protein - Protein Interaction (PPI) Network with the 7 genes associated with muscle function found over-expressed (Z-ratio above 1.96) shown in red. Interacting proteins are shown in yellow.

Table 2. Top ten GO categories of biological processes outstanding from the network.

G.O_ID	Description	p-value
16043	Actin filament polymerization	2.57E-14
9987	ATP synthesis coupled electron transport	1.58E-13
48518	Positive regulation of L-glutamate imports across plasma membrane	1.33E-12
10941	Negative regulation of apoptotic process	3.71E-12
22607	Actin filament bundle assembly	4.49E-12
43067	Negative regulation of cardiac muscle cell apoptotic process	8.01E-12
42981	Negative regulation of neuron apoptotic process	1.63E-11
48522	Activation of MAPKK activity	1.93E-11
44085	Contractile actin filament bundle assembly	3.00E-10
42221	Cellular response to calcium ion	7.76E-10

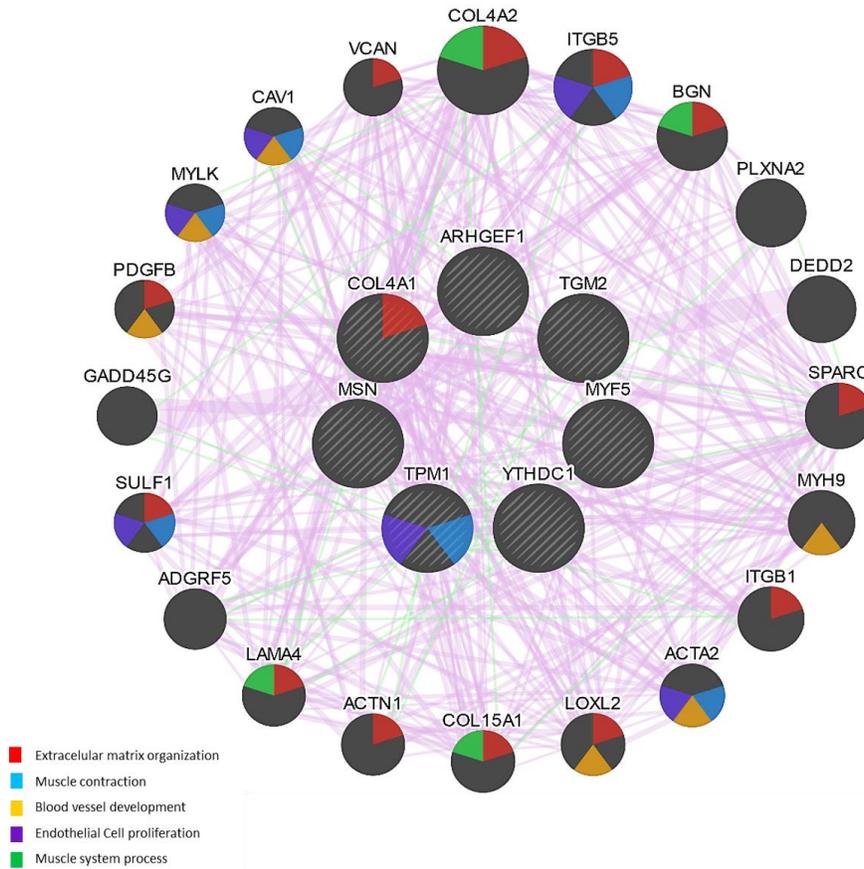*Note: p-values with Bonferroni correction are shown.*

Figure 2. Co-expression interaction network with the 7 genes associated with muscle function found over-expressed.
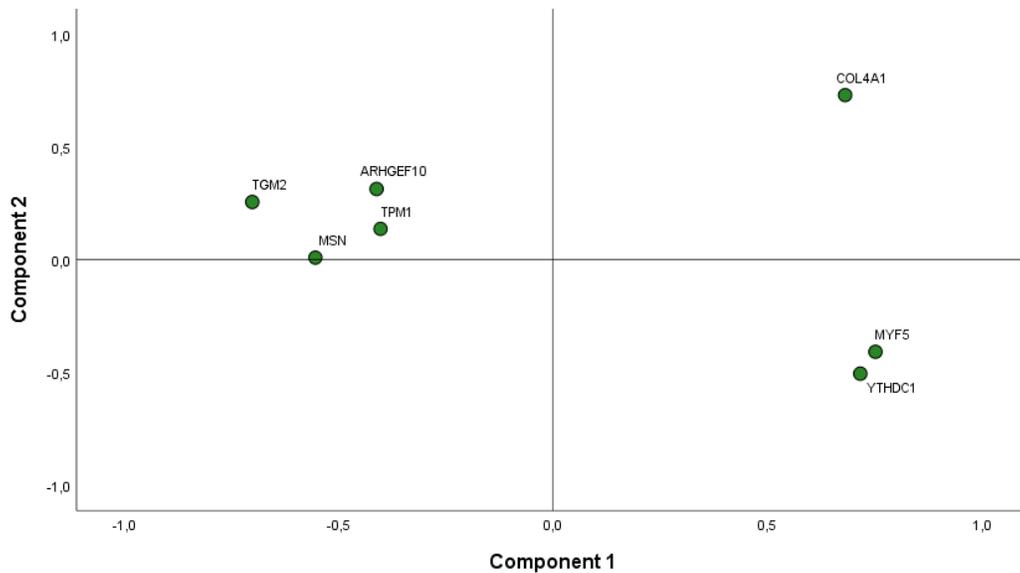

Figure 3. Pre-training samples PCA with the seven overexpressed genes shown in green.
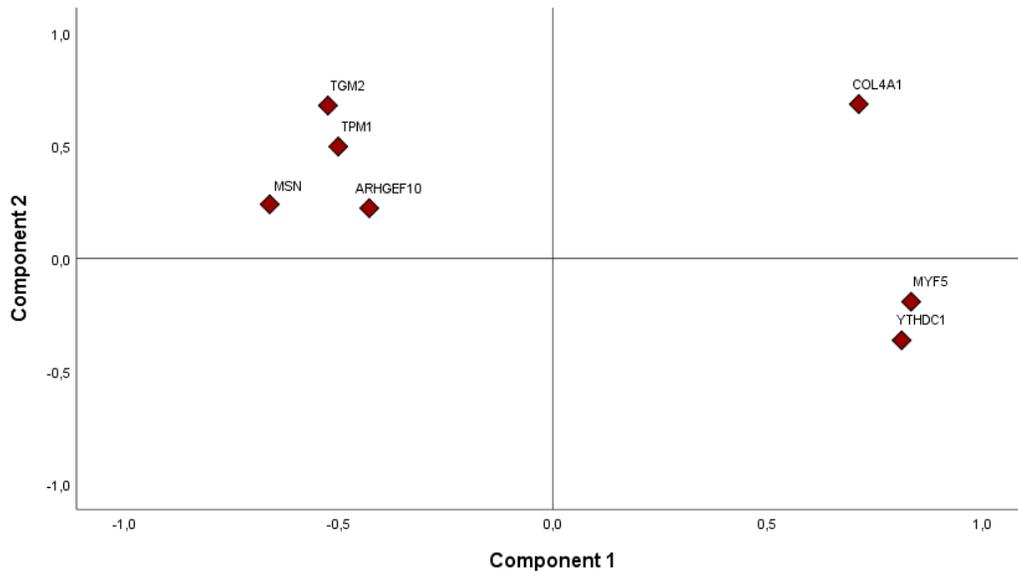
Figure 4. Post-training samples PCA with the seven overexpressed genes shown in red.

A 76% of the accumulated variance was reached within two components in the Pre-training samples PCA (Figure 3), while a 78% of the accumulated variance was reached within two components in the Post-training samples PCA (Figure 4).

DISCUSSION AND CONCLUSIONS

In the present study, we aimed to analyse the gene expression of genes associated with muscle function in vastus lateral samples, by performing bioinformatic analysis and identified expressed genes on publicly available after the exercise of an aerobic exercise for 20 weeks in human skeletal muscle.

The overexpressed genes were TPM1, COL4A1, MYF5, MSN, ARHGEF10, TGM2 and YTHDC1. These genes are known to be important in the structure / remodelling of the matrix / actin, as well as in mechano-transduction in skeletal muscle and any dysregulation may affect not only their function but also the one from the associated genes, as the PPI network shows. This is confirmed by the work of Turner et al. (2019), in which he performs a comparative transcriptome and analysis of methylome in the anabolism of human skeletal muscle, hypertrophy and epigenetic memory. They found 13 genes associated with muscular function in aerobic exercise, and two of them, MSN and COL4A1, are in fact, present in our study. It is confirmed that some of these genes have a structural relationship and a remodeller that increase after aerobic exercise and resistance in the expression of genes, protein abundance or activity level, such as; COL4A1 are involved in the regulation of muscle cells or satellites (Kanazawa, et al., 2019) (Xu, et al., 2017).

Adaptations of skeletal muscles dependent on frequency, duration and intensity (Egan & Zierath, 2013), the characterization of muscle proteins associated with different types of exercise are constantly replanted. Myofibrillar protein isoforms are the most frequent, mainly in the distribution of myosin heavy chain isoforms, thin filament isoforms, calcium proteins of the sarcoplasmic reticulum, mitochondrial proteins and some chaperones involved in the response to the correct one. In our study, the MYF5 gene was over-expressed, this gene acts as a transcriptional activator that promotes the transcription of muscle-specific genes and

plays a role in muscle differentiation. However, protein, activity, epigenetic factors, training activities during the performance of aerobic or glycolytic exercise, production in the muscle fibre, dysregulation in the distribution of the heavy chain of myosin, regulation of the low of the filament proteins in both the vastus lateralis and soleus muscles (Gonzalez-Freire, et al., 2017).

Moreover, mutations in type VI collagen (COL4A1) have shown abundant changes in proteins related to the cytoskeleton and sarcomere functions (Sela, et al., 2011), patients with skeletal muscle diseases such as hereditary myopathy of the body of inclusion, myofibrillary myopathies, desminopathies and some muscular disorders. These changes, not only to the skeletal muscle, but also to the connective tissues that produce contractions and weak muscle mutations in the genes that encode the sarcomeric and extraarcomeric proteins (De Palma, et al., 2014), for our study. COL4A1, at 20 weeks of training that contributes to proline 3-hydroxylation of collagen in the tendons, for a development of physical activity.

The most relevant GO categories found for the PPI network were mostly associated with muscular function, but we highlight the positive regulation of L-glutamate import across plasma membrane. L-glutamine is an amino acid that the body needs to stay in optimum health, and it has been demonstrated that during exercise, plasma glutamine levels increases and decreases, these variations are dependent on the type, duration, and intensity of exercise (Street, et al., 2011). The body naturally produces this amino acid, but it is also often consumed as a supplement in sports and bodybuilding as a supplement to help with muscle repair and growth, given the reports that show that L-glutamine might also help with post-exercise recovery (Street, et al., 2011) (Legault, et al., 2015). Legault et al. (2015), found that the supplementation with this amino acid resulted in faster recovery of peak torque and diminished muscle soreness following eccentric exercise of 16 healthy volunteers. The authors even concluded that the effect of L-glutamine on muscle force recovery may be greater in men than women.

Therefore, the present study demonstrates that skeletal muscle genes are regulated epigenetically by training loads for participants, in which DNA methylation is found, predominantly through hypomethylation and positive regulation of associated genes with the structure and remodelling of the matrix and the cytoskeleton. Similarly, the mechanics-transduction for skeletal muscle.

AUTHOR CONTRIBUTIONS

Mina, Matta and Zambrano wrote and verified the abstract and the introduction. Mina, Garcia and Rodriguez wrote and verified the methods, the results, the discussion and conclusions. All authors reviewed the final document and assisted in its construction.

SUPPORTING AGENCIES

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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